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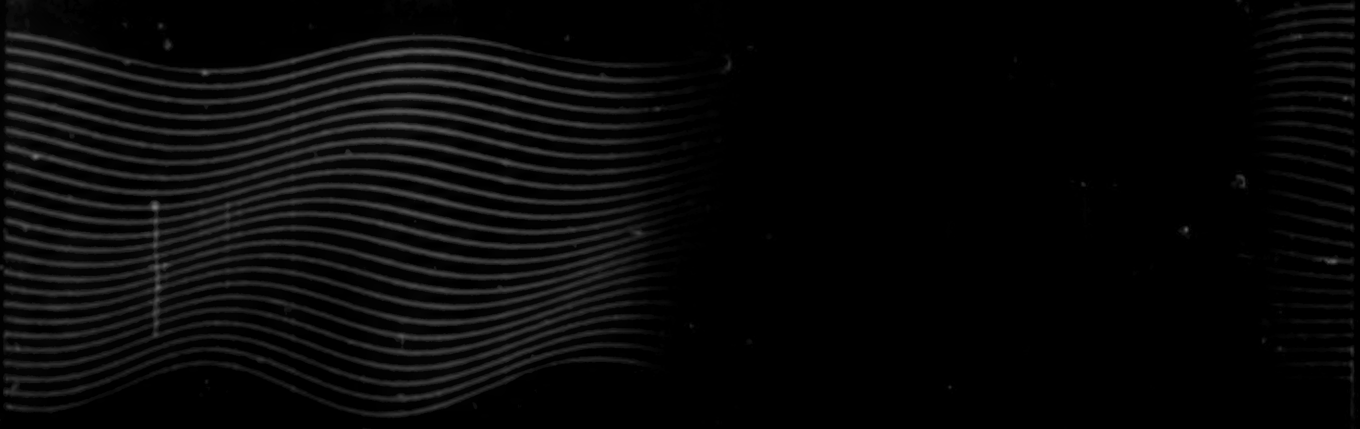

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Determination of optimal riparian forest buffer dimensions for stream biota–landscape association models using multimetric and multivariate responses

Emmanuel A. Frimpong, Trent M. Sutton, Kyoung J. Lim, Peter J. Hrodey, Bernard A. Engel, Thomas P. Simon, John G. Lee, and Dennis C. Le Master

Abstract: The dimensions of riparian buffers selected for stream biota–landscape association models determine correlation strength and subsequent model interpretation. Efforts have been made to optimize buffer dimensions incorporated into models, but none has explicitly determined a single optimum based on both longitudinal and lateral buffer dimensions. We applied partial correlation and multivariate linear regression on functional fish community response attributes and the index of biotic integrity using stream samples ($N = 107$) from the Eastern Corn Belt Plain Ecoregion of Indiana, USA. Land-cover data in digital format were processed in geographic information systems for an area covering 300 m on either side of selected streams and within 2000 m longitudinally. The optimal buffer dimension for the study area was 30 m laterally and 600 m longitudinally, with a partial correlation of 0.29 ($P = 0.002$), and there was agreement in the partial correlation and multiple regression models. The longitudinal dimension was more conclusively determined, but the lateral dimension was optimum only with respect to the resolution of the land-use data used. Based on these results, we propose the use of this approach to optimize the riparian buffer parameter in landscape models.

Résumé : La taille des bandes de protection retenue dans les modèles d'association des organismes et des paysages dans les cours d'eau détermine la force de la corrélation et l'interprétation subséquente du modèle. On a déployé beaucoup d'efforts afin d'optimiser la taille des bandes de protection utilisée dans les modèles, mais on n'a pas déterminé de façon explicite un optimum unique basé à la fois sur les dimensions longitudinales et latérales de la bande. Nous avons analysé à l'aide de corrélations partielles et de régressions linéaires multiples les caractéristiques des réponses fonctionnelles des communautés de poissons et les indices d'intégrité biotique dans des échantillons ($N = 107$) récoltés dans des cours d'eau de l'écorégion de la plaine de l'est de la ceinture de maïs en Indiana, États-Unis. Nous avons traité les données en format digital de couverture végétale à l'aide de systèmes d'information géographique sur une largeur de 300 m sur chaque rive des cours d'eau sélectionnés et sur une distance longitudinale de 2000 m. La taille optimale de la zone de protection dans la zone d'étude est de 30 m de largeur et de 600 m de longueur; la corrélation partielle est de 0,29 ($P = 0,002$) et les modèles de corrélation partielle et de régression multiple sont en accord. La dimension longitudinale est fixée de façon plus certaine, alors que la dimension latérale est optimale seulement compte tenu de la résolution des données d'utilisation des terres que nous avons employée. D'après ces résultats, notre recommandation est d'utiliser notre méthodologie pour déterminer les caractéristiques optimales des bandes de protection dans les modèles de paysages.

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Introduction

The increased recognition of landscape ecological concepts, in particular, the effects of scale on observed ecological phenomena (Wiens 1989), has resulted in a different view for stream ecology research. A number of investigations over the past two decades involving stream biota–habitat associations have extended beyond the stream channel to present a correlation of land cover in the watershed with either physical habitat or biotic variables measured at sample locations (e.g., Steedman 1988; Townsend et al. 2003; Wang et al. 2003). To demonstrate the influence of scale, many studies have compared the relative amount of variation in reach biotic or physical characteristics explained by land cover in the entire watershed with land cover somewhere in the vicinity of the sample location. While watershed land cover can be accurately quantified, the definition of an area within a watershed often is subject to ambiguity.

In defining the watershed area within the vicinity of a sampled reach, there are two potential approaches. The first approach considers land cover in the watershed within a certain radius of the sample location. However, a variation of this approach does not use a fixed radius but considers land cover in the area above a sample location and below the next sample location for sites nested in a larger watershed (e.g., Steedman 1988). The second approach considers land cover within a certain fixed distance from the stream channel, often called the riparian scale, which may be examined for a certain number of distances (e.g., Wang et al. 2003). Despite the recognition of the importance of both lateral and longitudinal dimensions of riparian areas, optimum dimensions have not been determined in a combined lateral and longitudinal approach. In reported studies that have looked at correlation of varying forested buffer areas with biotic attributes, one dimension of the buffer is usually fixed while the other is varied without explicitly stating the reason for choosing the fixed dimension. Changing the riparian area dimensions in a model may lead to increased or decreased correlation between forested riparian area and biotic attributes, which could alter model interpretation.

Among landscape ecologists who study streams, it is an unresolved question whether whole watershed or riparian land cover has a greater influence on streams and their biota (Rabeni and Sowa 2002; Wang et al. 2003; Allan 2004). To attempt to answer this question, the dimensions of riparian area used in analysis cannot be chosen arbitrarily. Modelers intending to use forested buffer as an explanatory variable for variation in stream biotic attributes can begin with a heuristic search using plausible subwatershed areas to determine the optimum dimension for the geographic area where the model is to be applied. This is a way to define riparian area if the relative influence of riparian and watershed land cover, for example, are to be compared. Because watersheds differ in size, a logical approach to finding a common riparian dimension is to remove the influence of size from the response variable. Alternatively, an interaction of watershed size with riparian forest influence may be assumed to exist and be incorporated in statistical tests. The objectives of this study were (*i*) to determine the lateral and longitudinal dimensions of forest buffer that is most correlated with the index of biotic integrity and its metrics in the Eastern Corn Belt Plain Ecoregion of Indiana, and (*ii*) to demonstrate how the methods used can be generalized to other landscapes and similar modeling efforts involving stream biota – land cover relationships.

Methods

Study area and sampling

We analyzed the association between percent of natural vegetation (called riparian forest or buffer hereafter) and fish assemblage characteristics for 107 stream locations within the approximately 44 000 km^2 Eastern Corn Belt Plain (ECBP) Ecoregion of Indiana. Land use in the ECBP Ecoregion is greater than 75% row-crop agriculture in a predominantly corn – soy bean rotation. Fifty-seven fish assemblage samples were collected by one-pass backpack electrofishing from 1990 through 1994, and 50 additional samples were collected in 2002 and 2003 from reaches that ranged in length from 15 to 20 mean stream widths from June to September. Sampled streams were mostly low-gradient, first- to fifth-order streams, with 0.06%–1.92% main channel slopes (0.23% average) and watershed size ranging from 3.1 to 269.5 km^2. Fish collected were counted, identified to species, and classified into trophic, reproductive, and sensitivity guilds following Simon and Dufour (1998). The multimetric index of biotic integrity (IBI) scores were computed for sites using metric expectations developed by Simon and Dufour (1998). Nine IBI metrics that were correlated with various dimensions of forested buffer were selected to be included in a multivariate model. The selected metrics were number of sensitive species, number of sucker (family Catostomidae) species, number of headwater species, number of darter (Etheostomatini), madtom (*Noturus*), and sculpin (*Cottus*) species, percent abundance of tolerant individuals, proportion of pioneer species, proportion of omnivores, proportion of simple lithophilic spawners, and catch-per-unit-effort (CPUE). The remaining IBI metrics were species richness, number of darter (Etheostomatini) species, number of sunfish species, proportion of insectivorous individuals, number of minnow species, proportion of carnivorous individuals, and proportion of individuals with deformities, eroded fins, lesions, and tumors.

Spatial data computations

United States Census Bureau streams were imbedded into the US Geological Survey (USGS) 30-m digital elevation model (DEM) and used to delineate watersheds for all sample locations and generate stream networks using the Watershed Delineator extension in ArcView 3.3 (ESRI, Redlands, California). We used a threshold area of approximately 0.5 km^2 to define a stream. To determine land cover in watersheds near the time of sampling, the following two sources of 30-m resolution digital land-cover data were used: the National Land Cover Data (sites sampled from 1990–1994) and the National Agricultural Statistical Service crop-use data (sites sampled in 2002 and 2003). Land-cover grids were reclassified as forest, agricultural, or developed and converted to vector data. Around each sample location, 10 concentric buffer polygons established 200 m apart were created, and an additional 10 buffer polygons were created 30 m apart around the entire stream network. These two sets of polygons were intersected together with the vectorized land-cover data and watershed boundary, thereby enabling the computation of the cumulative percent of forest in each of 100 combinations of buffer dimensions within each watershed. The buffer dimensions were up to 2000 m in steps of 200 m longitudinally upstream and up to 300 m in steps of 30 m laterally on either side of the stream. It was believed that this range covered the optimum buffer dimension based on studies such as Barton et al. (1985) and Steedman (1988). Because of the large amount of data extractions involved, most of the overlay and computations were automated in ArcView using customized Avenue scripts.

Statistical models

Fish assemblage – riparian buffer associations were investigated by two underlying statistical models, with all analyses performed in SAS® 8.02 (SAS Institute Inc., Cary, North Carolina). The first model was a first-order partial correlation of IBI and percent forested buffer evaluated by partialing out the effect watershed area. The partial correlation model is described by Neter et al. (1996) and the analysis was available in SAS through the PARTIAL statement of the CORR procedure. In the application of this model to three variables, Y_1 was IBI score at the sample location, Y_2 was $\log_{10}$(1 + percent forested buffer of a given lateral and longitudinal dimension), and Y_3 was $\log_{10}$(watershed size). The dimensions of the riparian buffer used to compute Y_2 were varied to find the combination that maximized the partial correlation coefficient.

The second model was a multivariate linear regression in which simultaneous significance tests on the relationship between explanatory variables and more than one response variable may be obtained. The simultaneous test is only as good as the common trait of the system being modeled by the response variables together (Finn 1974; Johnson and Wichern 2002). For this model, consider m responses $Z_1, Z_2, \ldots, Z_m$ and a single set of predictor variables $Y_1, Y_2, \ldots, Y_r$. The multivariate linear regression model is

$$(1)\qquad \underset{(n\times m)}{\mathbf{Z}} = \underset{(n\times(r+1))}{\mathbf{Y}}\ \underset{((r+1)\times m)}{\boldsymbol{\beta}} + \underset{(n\times m)}{\boldsymbol{\epsilon}}$$

where $\mathbf{Z}$ is a matrix of response variables, $\mathbf{Y}$ is the matrix of predictors, $\boldsymbol{\beta}$ is the matrix of parameters, and $\boldsymbol{\epsilon}$ is a matrix of error terms. Each Z_i follows the linear regression model. Finn (1974) provides the theory of the test of simultaneous hypotheses on rows of $\boldsymbol{\beta}$. These tests were available in SAS through the MTEST statement in the REG procedure. The model was applied in this study using nine response variables for each of 107 sites. For the $\mathbf{Z}$ matrix, the variable Z_1 was the number of sensitive species, Z_2, the number of sucker (family Catostomidae) species, Z_3, the number of headwater species, Z_4, the number of darter (Etheostomatini), madtom (*Noturus*), and sculpin (*Cottus*) species, Z_5, the percent abundance of tolerant individuals, Z_6, the proportion of pioneer species, Z_7, the proportion of omnivores, Z_8, the proportion of simple lithophilic spawners, and Z_9, CPUE. The $\mathbf{Y}$ matrix had the following three variables: Y_2 and Y_3 (defined in the previous paragraph) and the multiplicative term Y_2Y_3 representing the interaction between forested buffer and watershed area. The hypotheses that the parameters for the predictors Y_2 and Y_2Y_3 are zero were tested simultaneously. The interpretation of the hypotheses together is that neither riparian forest nor its interaction with watershed area has a significant relationship with any of the response variables Z_1 to Z_9. The dimensions of the riparian buffer were varied to find the region of minimum probability of type-I error for rejecting the simultaneous hypothesis.

Response visualization

Because the riparian buffer was two-dimensional, the response of fish community attributes to varying buffer dimensions was best visualized as a surface. No detailed theory was applied in fitting the response surface. Simple linear interpolations of the partial correlation coefficient (for the first model) and the probability of a type-I error (for the second model) were performed. Procedures described by Cohen et al. (1998), and available through SAS/INSIGHT® (SAS Institute Inc.), provided quick contour plots to visually determine the approximate optimal buffer dimensions for the study area. The graphing software SigmaPlot® (Systat Software Inc., Point Richmond, California) provided more editing functionality and was used to create the plots included in this report.

Results and discussion

The IBI scores ranged from 16 to 56 with an average of 38 (maximum score is 60), and the percent of forested area in buffers ranged from 0% to 100% in all combinations of lateral and longitudinal distances. The partial correlation between IBI scores and the percent of forested riparian buffer was maximized at 600 m in the longitudinal (upstream) direction and peaked laterally at closest to the stream with a partial correlation of 0.29 ($P = 0.002$) (Fig. 1). The multivariate test also had a minimum probability of type-I error centered on 600 m longitudinally and below 60 m (Fig. 2). If a 30 m or 60 m × 600 m buffer dimension is selected, the probability of a type-I error is less than 1% if we reject the hypothesis that neither percent forested buffer nor its interaction with watershed area has a significant relationship with any of the nine fish assemblage attributes. The lateral pattern of partial correlation between 30 m and 300 m shows that at any fixed longitudinal dimension, the lateral dimension nearest the stream has the strongest correlation. However, the change in correlation is not linear and decreases steeply away from the stream, leveling off at 150 m. At a fixed interval, the statistical significance of the difference in correlation between two lateral dimensions was improbable the greater the distance they are from the stream.

Considering the behavior of the partial correlation and the probability of a type-I error around the optimum point, it is unlikely that there are other optima within the watershed. Barton et al. (1985) similarly found that the optimal stream bank distance upstream needed to explain weekly mean temperature and the distribution of trout species in southern Ontario streams was approximately 1 km. This trend is also consistent with Steedman's (1988) observation that partial basin land use provided better predictive power of regression models for IBI, particularly with increasing mean watershed size. It appears that for agricultural areas, the influence of forest on fish communities is localized. The optimum buffer dimension based on our data resolution was approximately

Fig. 1. Response of the partial correlation coefficient for the index of biotic integrity (IBI) with percent forested riparian buffer for a given lateral and longitudinal buffer dimensions upstream of sample locations. The correlation was evaluated by partialing out the effect of watershed size on IBI for 107 first- to fifth-order streams of the Eastern Corn Belt Plain Ecoregion of Indiana, USA.

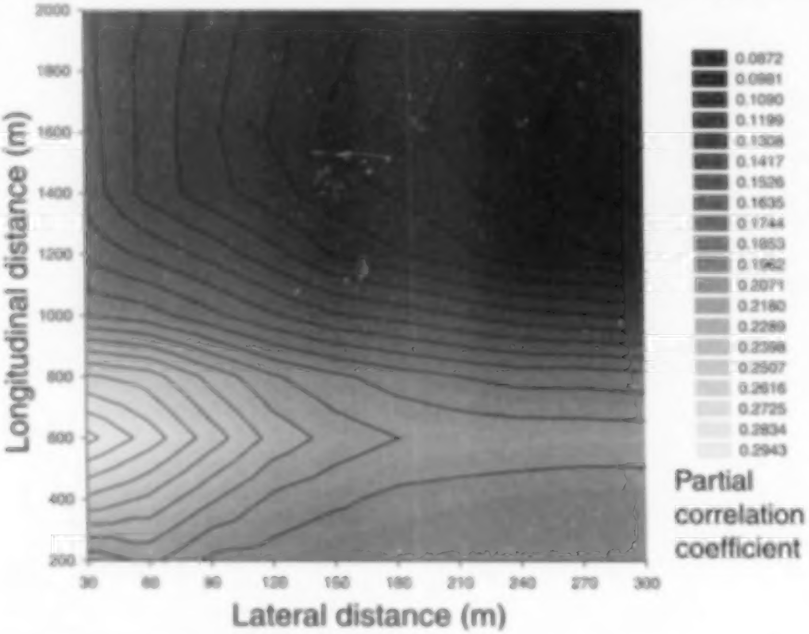
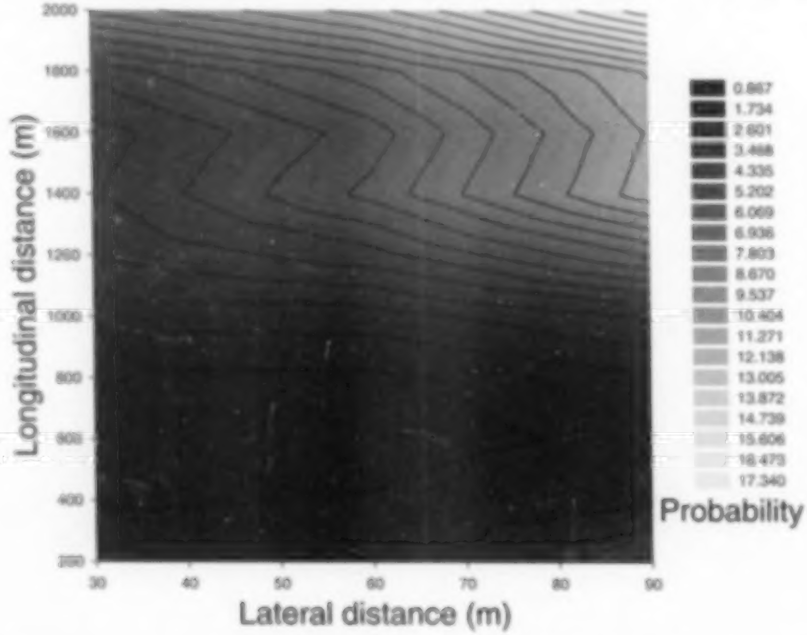


Fig. 2. Probability of type-I error for rejection of the null hypothesis that neither the percent forested riparian buffer nor its interaction with watershed size has a relationship with nine fish community attributes for a range of lateral and longitudinal buffer dimensions upstream of sample locations. The 107 streams of the Eastern Corn Belt Plain Ecoregion of Indiana, USA, varied from first to fifth order.



30 m × 600 m. Below this optimum, insufficient information has been included, and beyond the optimum, too much information has been included, with both leading to weaker correlations or higher probability of error in the choice of buffer dimensions. There is a caveat in our choice of the lateral optimum in that we do not know what the trend would

be laterally at dimensions less than 30 m. It is tempting to extrapolate the lateral trend to the stream and suggest that correlations should increase to the stream bank. Aerial photos would permit such fine-resolution investigations. In this study, we used land cover at the time of sampling; however, similar analyses could be performed with historical land cover if that is deemed more relevant to the model at stake. We obtained similar results using both multimetric and multivariate response variables, but the multivariate approach is considerably more complex. In real-world applications, the multivariate approach will be more useful when more than one biotic variable or combinations of variables (for example, IBI and an invertebrate community index or a mixture of metrics from these two indices) are under consideration for a single model.

It should be pointed out that while this method leads to identification of the zone of highest correlation between some landscape variable and stream biotic attributes, the variation accounted for by a given landscape variable may be small and require that additional or alternative landscape attributes be investigated. For instance in this study, the 30 m × 600 m forested buffer accounted for only about 10% of the variation in IBI at the ecoregional scale. In a related study, another landscape variable, a combined lengths and steepness of slopes factor, within a 300 m × 1000 m zone accounted for more variation in IBI than forested buffer in the ECBP Ecoregion. The 30 m × 600 m forested buffer assumed greater importance in smaller watersheds within the ecoregion, accounting for 50%–60% of the variation in IBI in watersheds one-third to one-quarter of the size of the ECBP Ecoregion, where surficial geology was more homogenous. We observed that as the spatial extent of a model was expanded, less biotic variation was accounted for by forested buffer. We attribute this to increased heterogeneity of surficial geology over a large area.

This study employed a new approach to modeling stream biota – land use association. The methodology is applicable to modeling in any geographic location and any biotic response. Stream landscape models have been developed based on ecoregions or watersheds as the spatial unit and response variables have been predominantly fish and macroinvertebrate community attributes. Political boundaries are less meaningful as spatial units, and stream physical and chemical habitat variables are not the ultimate response following landscape modification. Riparian landscape alteration causes changes in the abundance and composition of the aquatic biota, mediated by the changes in physical and chemical habitat and energy sources over what appears to be a short distance (about a kilometre or less). Forested reaches typically have cooler temperatures, wider channels, and fewer sediments (Allan 2004). The effectiveness of the ecosystem functions performed by riparian vegetation is affected by landform (slopes, geology, etc.) and location (Rabeni and Sowa 2002; Allan 2004). The question of where and how much of landscape alteration will cause a hypothesized change in fish or macroinvertebrate community attributes can be explored using the methods of this study. Researchers interested in specific functional groups can focus on those groups. It is generally agreed that different stream fauna respond to landscape processes at different scales. Our methodology provides a means for evaluating different species and functional groups in different landscape settings to corroborate this generally held view. To apply this method, all one needs is spatially referenced biological sample locations and digital land-cover and elevations data. Large sample sizes are recommended owing to the inherent variability in biological systems. The choice of response variables should be determined by the management question posed. Specific species – land cover relations would be appropriate in systems dominated by one or a few species but will have limited practical use in multispecies systems. This method will be more useful in landscapes dominated by one of two land-cover types, agricultural or natural vegetation, where physical processes linking landscapes and streams are diffuse and effects of vegetation or agricultural runoff decline with distance from the upstream source. Our specific findings apply to the ECBP Ecoregion and perhaps similar agricultural landscapes where forests are located predominantly in floodplains.

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Generalized instream habitat models

Nicolas Lamouroux and Ian G. Jowett

Abstract: Conventional instream habitat models (e.g., the physical habitat simulation system) predict the impact of regulation on the habitats of freshwater taxa. They link a hydraulic model with microhabitat-suitability models for taxa to predict habitat values at various discharge rates. Their use requires considerable field effort and experience. Recent analyses performed in France suggested that comparable results could be achieved using simplified hydraulic data. We tested this approach for 99 stream reaches and nine aquatic taxa in New Zealand. The resulting generalized habitat models predict habitat values similar to those predicted by conventional models from simplified hydraulic data (depth–discharge and width–discharge relationships, average particle size, and mean annual discharge). As in France, within-reach changes in habitat values were linked to the specific discharge of reaches, while between-reach changes depended mainly on the Froude number at mean annual discharge. The generalized models perform well outside their calibration range. Models previously developed in France perform well in New Zealand. Such generalized models contribute to identifying the key hydraulic variables for freshwater taxa and should facilitate habitat studies worldwide.

Résumé : Les modèles d'habitat conventionnels comme « physical habitat simulation system » prédisent les impacts des aménagements sur l'habitat des organismes aquatiques. Ils couplent un modèle hydraulique avec des modèles de préférences biologiques, pour prédire des valeurs d'habitat en fonction du débit. Des modèles alternatifs moins coûteux ont été récemment développés en France. Nous testons cette approche alternative pour neuf taxons sur 99 tronçons de rivières de Nouvelle-Zélande. Nous développons ainsi des modèles d'habitat généralisés qui donnent des estimations fiables des valeurs d'habitat à partir de variables hydrauliques simples (lois hauteur–débit et largeur–débit, substrat moyen et débit journalier moyen). Comme en France, les valeurs d'habitat varient essentiellement avec le débit spécifique intra-rivière et avec le nombre de Froude (au débit moyen) inter-rivières. Les modèles restent généralement pertinents hors de leur domaine de validation. Les modèles auparavant développés en France restent valides en Nouvelle-Zélande. Ces modèles généralisés nous indiquent les variables hydrauliques clés pour les organismes lotiques. Leur généralité devrait faciliter les études d'habitat hydraulique dans le monde.

Introduction

Instream habitat models became popular and yielded a wealth of instream flow studies in the last two decades (Reiser et al. 1989; Gallagher and Gard 1999; Guay et al. 2000). They are attractive for quantifying the impacts of morphological (e.g., channelization or weir construction) or hydrological (changes in discharge regime, minimum flow, flooding properties) modifications of rivers.

Conventional instream habitat models (e.g., the physical habitat simulation system (PHABSIM), Bovee 1982; the river hydraulics and habitat simulation system (RHYHABSIM), Jowett 1989; the système d'evaluation de l'habitat physique (EVHA), Ginot 1998) link a traditional hydraulic-engineering model to habitat-suitability curves for water depth, velocity, bed particle size, and other habitat variables (e.g., cover). The hydraulic model predicts the values of point habitat variables (water-column velocity, depth, particle size) as a function of the discharge in individual cells within a stream reach. Suitability curves are used to transform point habitat variables into point habitat values (ranging between 0 and 1) in each cell. A weighted usable area (WUA) is computed at the reach scale as the sum (across cells) of point habitat values multiplied by the area of the cell. A reach habitat value (HV, ranging between 0 and 1) can be calculated as WUA divided by the wetted area of the reach. Therefore, the major reach-scale output of conventional instream habitat models consists of changes in HV or WUA with discharge rate.

Applying conventional instream models in a stream reach requires considerable field effort and experience. It involves a complete survey of bed topography and precise measurements of current velocities and water depths along a number of georeferenced cross sections, depending on the form of hydraulic model. The hydraulic model also requires calibration. Several approaches have been proposed for reducing this effort. Some are based on a simplification of the hydraulic complexity within the reach by, for example, regarding

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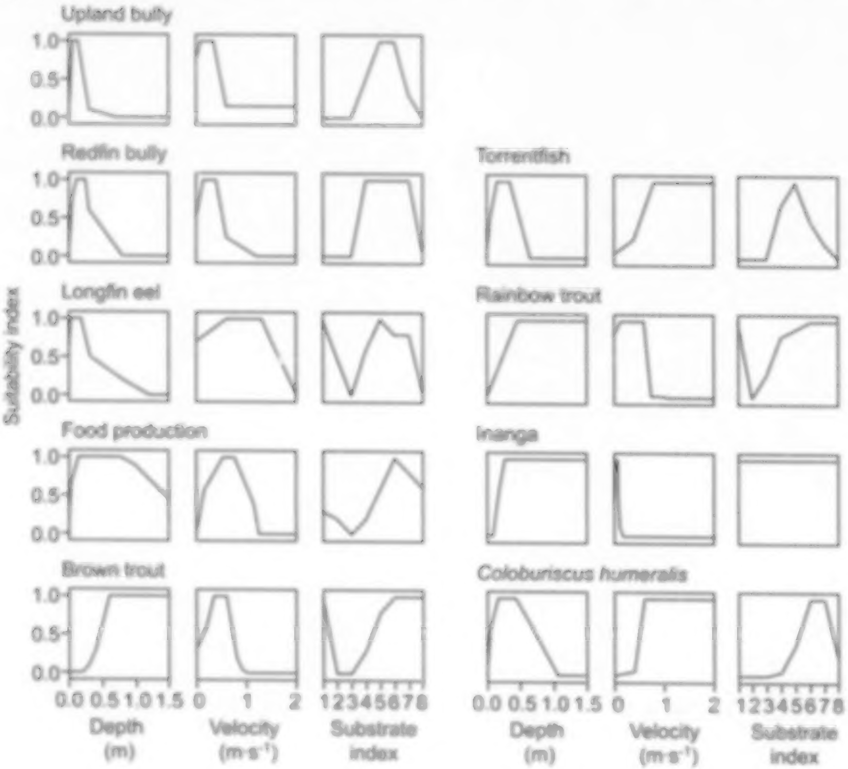
Fig. 1. The microhabitat-suitability curves used. Substrate indices 1–8 represent vegetation, silt, sand, fine gravel, gravel, cobble, boulder, and bedrock, respectively.



point velocities as equal to their average (Jowett 1998), or simplifying their statistical distribution (Singh and Broeren 1989; Rao et al. 1993; Lamouroux et al. 1998). Others try to identify general patterns in existing applications of the models (Hatfield and Bruce 2000). Lamouroux and Capra (2002) proposed to model directly the output of conventional instream habitat models using simplified and cost-effective reach descriptions (depth–discharge and width–discharge relationships, particle size, median flow). The resulting generalized habitat models do not require any simplifying hypothesis concerning the distribution of hydraulic variables within reaches. Their use requires little experience and field effort, and the models provide HV and WUA curves that are interpreted similarly to conventional ones.

In this paper, we test the approach of Lamouroux and Capra (2002) in New Zealand rivers and for New Zealand taxa. In addition, we test whether the generalized models previously developed in France remain valid in the different geographic context of New Zealand. Specifically, we collected 99 existing applications of conventional instream habitat models (RHYHABSIM; Jowett 1989) in a wide range of streams in New Zealand. We ran RHYHABSIM to estimate HVs for nine freshwater taxa and average reach characteristics (depth, width, particle size) at different discharge rates. HV was chosen as the variable to model because it depends less on stream size than WUA does. We used parametric modeling to build generalized habitat models explaining HV variations (both within and between reaches) from average reach characteristics. We tested the generalized models in 14 larger or smaller stream reaches that were not used in developing the generalized models. We also ran RHYHABSIM using microhabitat-suitability curves for French species, and tested whether the generalized models previously developed in France predicted the results in New Zealand rivers correctly.

Methods

We used methods similar to those used by Lamouroux and Capra (2002) and Lamouroux and Souchon (2002). We describe them briefly in this paper.

Taxa considered and suitability curves

We used existing microhabitat-suitability models for nine New Zealand habitat uses covering a wide range of taxa and habitats. These were conventional suitability curves for point velocity, water depth, and particle size. They give, for each habitat variable, a suitability index varying between 0 and 1 (Fig. 1). We considered habitat suitability for four benthic fish species or specific life stages: upland bully (*Gobiomorphus breviceps*), redfin bully (*Gobiomorphus huttoni*), juvenile (<30 cm long) longfin eels (*Anguilla dieffenbachii*), and torrent fish (*Cheimarrichthys fosteri*). Upland bullies are typically found in shallow and slow-flowing water, juvenile eels in water of a wide range of depths and velocities, redfin bullies in water of moderate velocities and in association with boulders, and torrentfish in swift-flowing riffles up to 0.5 m deep (Jowett and Richardson 1995). Suitability curves for three nonbenthic life stages of fish and two benthic invertebrate taxa were also considered: feeding adult brown trout (*Salmo trutta*) (Hayes and Jowett 1994), adult rainbow trout (*Oncorhynchus mykiss*) (Raleigh et al. 1984), feeding inanga (*Galaxias maculatus*) (Jowett 2002), a mayfly (*Coloburiscus humeralis*) (Jowett 2000), and benthic invertebrates in general ("food production" as described by Waters (1976)). Bullies, torrent fish, and inanga are relatively small fish (<150 mm long) and habitat-suitability curves do not distinguish between different size classes or life stages (Glova and Duncan 1985; Jowett and Richardson 1995).

We also used suitability curves for specific life stages of three French cyprinids: adult barbel (*Barbus barbus*), adult stone loach (*Barbatula barbatula*), and adult minnow (*Phoxinus phoxinus*). The barbel typically prefers fast and deep microhabitats with large particles, the loach is a riffle-dwelling species, and the minnow prefers intermediate velocity and water depth. French suitability curves were derived from electrofishing data and are those used and shown in Lamouroux and Capra (2002).

Stream reaches

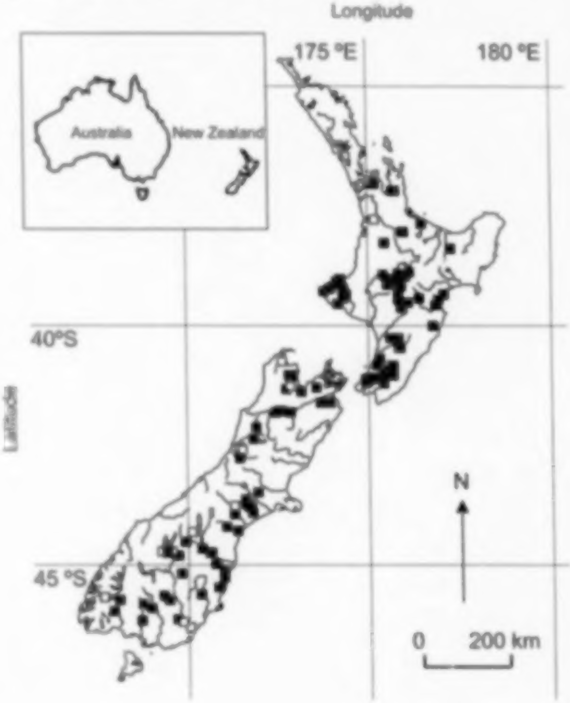
We estimated HVs and average reach characteristics in a total of 99 reaches in 86 rivers in New Zealand, 51 in the North Island and 48 in the South Island (Fig. 2). Although the flows in some of these reaches have been modified, their channel morphologies have not been affected. They cover a variety of geomorphological and hydrological contexts (e.g., from rivers of low flow variability because of frequent rainfall or water storage in lakes or groundwater to rivers with infrequent rainfall and high flow variability). Reach length was, on average, 13.7 times water-surface width. Mean annual discharges of the reaches ranged between 0.6 and 54 $m^3 \cdot s^{-1}$, average widths between 3.6 and 76 m, average water depths between 0.14 and 1.3 m, and average particle size between 1.3 and 34 cm.

Running habitat models to obtain HVs and average reach characteristics

Habitat modeling was performed using RHYHABSIM software (Jowett 1989), using principles similar to those of PHABSIM (Bovee 1982). RHYHABSIM combines a one-dimensional hydraulic model that estimates the stage–discharge relationship at different cross sections, a conveyance procedure that estimates the point velocities along each cross section, and the suitability curves described above. Model input consists of a detailed topography of the stream reach associated with velocity/depth/particle-size measurements made along several (between 8 and 68, average 23) cross sections. The survey data were collected by a wide variety of teams for research or management purposes.

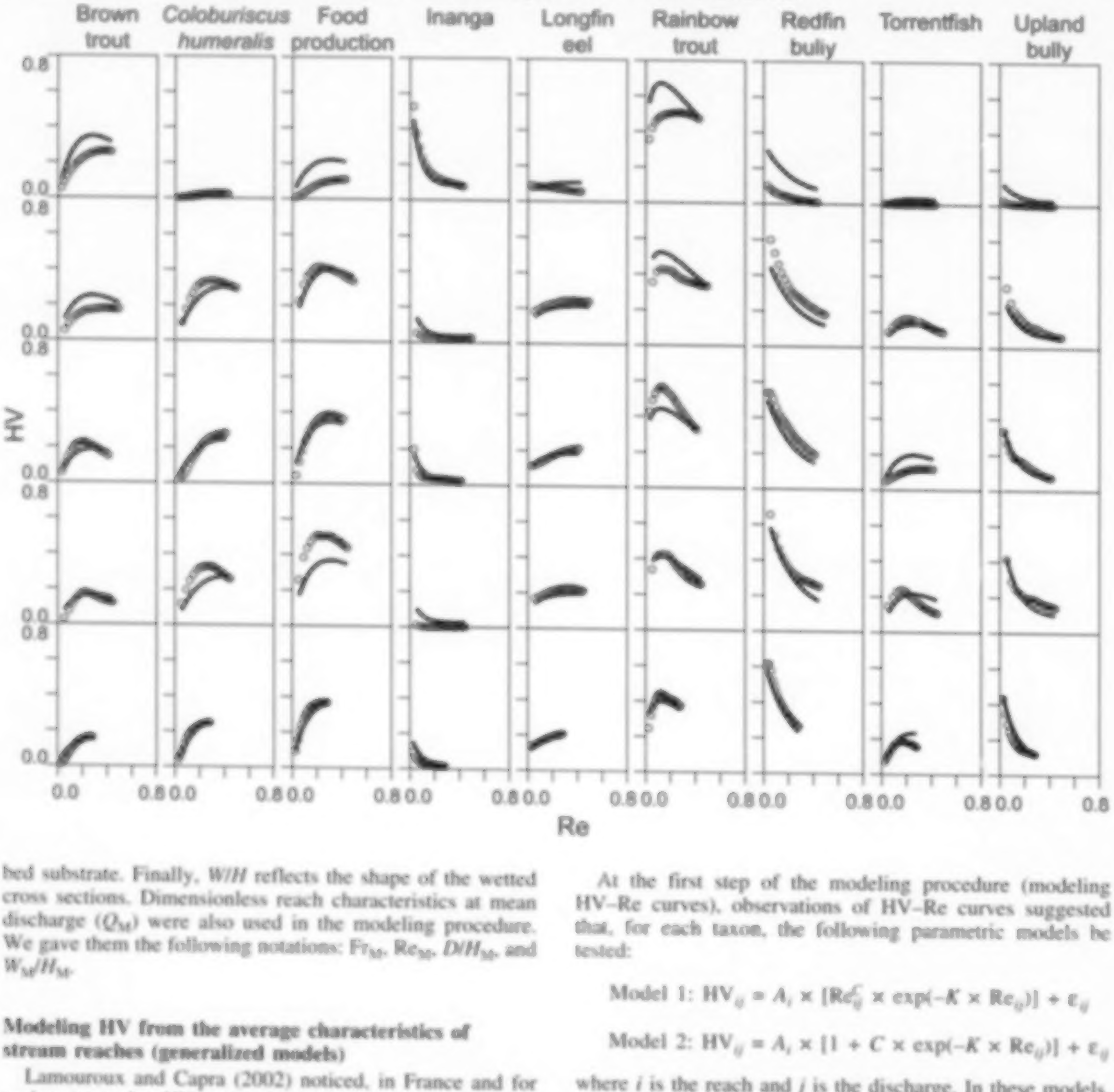
Running RHYHABSIM for each reach provided HVs at 20 different discharges. Discharge ranged between $Q_M/20$ and Q_M, where Q_M is the mean natural discharge. Q_M was estimated using 1–103 years of discharge data collected at permanent gauging stations, with an average record length of 27 years. Gauges were relatively close to the reaches (<10 km), thereby minimizing potential bias caused to Q_M by small tributaries between the studied reaches and the gauges.

Fig. 2. Locations of the 99 stream reaches in New Zealand that were used to generate the generalized models (■) and the 14 reaches used for validation (□).



RHYHABSIM was also used to calculate the average hydraulic characteristics of reaches, at the same 20 discharge values as HV. Average hydraulic characteristics were the mean wetted width (W) and mean depth (H) at varying discharges (Q). The mean particle size (D) was also estimated from survey data as the mean of point values of particle size weighted by the area they represented. Note that the reach-averaged velocity was not included because it can be estimated as $Q/(WH)$. We used dimensionless combinations of the variables listed above to describe average reach characteristics in the modeling procedure (following Lamouroux and Capra 2002): Froude number (Fr), Reynolds number (Re), relative roughness (D/H), and width to depth ratio (W/H). Fr is a ratio between kinetic and gravitational forces, and classifies flows from subcritical (Fr < 1) to supercritical (Fr > 1). In terms of habitats, it reflects the proportion of riffles versus pools in reaches, because it has contrasting values in these habitat types (Fr is generally <0.2 in pool habitat and >0.4 in riffle habitat; Jowett 1993). Re, as calculated herein (note that Re is multiplied by 10^{-6} throughout the paper; see List of symbols), is a specific discharge (discharge per unit width). It is therefore sensitive to flow rate in reaches. Re also quantifies the level of turbulence. D/H quantifies the amount of shelter and roughness provided by

Fig. 3. Observed habitat values (HVs) versus specific discharge (Reynolds number (Re)) curves (outputs of the river hydraulics and habitat simulation software (RHYHABSIM; ○) and modeled curves from average characteristics of reaches (using our generalized habitat models (solid lines)) for 5 reaches selected at random among the 99 used to build the model.



bed substrate. Finally, W/H reflects the shape of the wetted cross sections. Dimensionless reach characteristics at mean discharge (Q_M) were also used in the modeling procedure. We gave them the following notations: Fr_M, Re_M, D/H_M, and W_M/H_M.

Modeling HV from the average characteristics of stream reaches (generalized models)

Lamouroux and Capra (2002) noticed, in France and for various taxa, that HV versus Re curves had somewhat similar shapes in all reaches but different values overall. A multivariate analysis of all HVs, not shown for simplicity, also confirmed that Re was the best explanatory variable for reflecting common patterns (among reaches) in HV variations in New Zealand rivers (see also Fig. 3). Therefore, we modeled HV–Re curves in two steps for each species. As a first step, we modeled HV–Re curves as the product of a similar functional form (shared by all reaches) and a reach parameter (which varied among reaches). As a second step, we explained the parameter variations among reaches from reach characteristics at Q_M.

At the first step of the modeling procedure (modeling HV–Re curves), observations of HV–Re curves suggested that, for each taxon, the following parametric models be tested:

$$\text{Model 1: } \mathrm{HV}_{ij} = A_i \times [\mathrm{Re}_{ij}^{C} \times \exp(-K \times \mathrm{Re}_{ij})] + \varepsilon_{ij}$$

$$\text{Model 2: } \mathrm{HV}_{ij} = A_i \times [1 + C \times \exp(-K \times \mathrm{Re}_{ij})] + \varepsilon_{ij}$$

where i is the reach and j is the discharge. In these models, C and K are parameters that are shared by all stream reaches (they are fitted to mimic the common shape of the changes in HV with Re between reaches). In contrast, A is a reach scaling factor that indicates the overall level of HVs in a given reach. The error term (ε_{ij}) is a random deviation. Model 1 can reproduce a wide range of bell-shaped curves. It predicts a HV of 0 at Re = 0, a maximum HV at Re = C/K, and a decrease in HV for higher Re values. Model 2 can reproduce an exponential increase or decrease in HV with increasing Re values.

Parameters A, C, and K were fitted using nonlinear mixed-effects models (for mathematical bases see Lindstrom and

Table 1. Generalized habitat models used to predict habitat values (HVs) from average characteristics of stream reaches.

Taxon	Model	C	K	Model for A	Variance within reaches (%)	Variance in all reaches pooled (%)
Brown trout, *Salmo trutta* (adult)	1	0.93	3.34	$0.057 - 1.69\ln(\mathrm{Fr_M}) + 0.84\ln(\mathrm{Re_M}) + 2.5\,D/H_M$; $r^2 = 0.71$, $P < 0.001$	74	73
Coloburiscus humeralis	1	1.10	2.77	$3.05 + 1.57\ln(\mathrm{Fr_M}) + 3.41\,D/H_M - 0.41\ln(\mathrm{Re_M})$; $r^2 = 0.52$, $P < 0.001$	78	61
Food production	1	0.85	2.73	$2.74 + 0.70\ln(\mathrm{Fr_M}) + 2.23\,D/H_M$; $r^2 = 0.26$, $P < 0.001$	76	54
Inanga, *Galaxias maculatus* (feeding)	2	6.97	15.76	$-0.017 - 0.055\ln(\mathrm{Fr_M}) + 0.013\ln(\mathrm{Re_M})$; $r^2 = 0.65$, $P < 0.001$	81	80
Longfin eel, *Anguilla dieffenbachii* (<30 cm)	1	0.31	0.78	$0.415 + 0.128\ln(\mathrm{Fr_M}) + 0.34\,D/H_M - 0.027\ln(\mathrm{Re_M})$; $r^2 = 0.54$, $P < 0.001$	68	58
Rainbow trout, *Oncorhynchus mykiss* (adult)	1	0.27	2.2	$0.44 - 0.654\ln(\mathrm{Fr_M}) + 0.299\ln(\mathrm{Re_M}) + 0.859\,D/H_M$; $r^2 = 0.76$, $P < 0.001$	70	76
Redfin bully, *Gobiomorphus huttoni*	2	6.96	4.64	$0.075 + 0.018\ln(\mathrm{Fr_M}) + 0.0004\,W_M/H_M$; $r^2 = 0.48$, $P < 0.001$	88	72
Torrent fish, *Cheimarrichthys fosteri*	1	0.86	3.39	$2.88 + 1.58\ln(\mathrm{Fr_M}) - 0.83\ln(\mathrm{Re_M}) - 2.45\,D/H_M$; $r^2 = 0.72$, $P < 0.001$	71	72
Upland bully, *Gobiomorphus breviceps*	2	5.85	8.67	$0.064 + 0.0006\,W_M/H_M + 0.027\ln(\mathrm{Fr_M}) - 0.01\ln(\mathrm{Re_M}) - 0.035\,D/H_M$; $r^2 = 0.70$, $P < 0.001$	87	79

Note: "Model" indicates the parametric model providing the best fit (model 1 or 2; see the text for equations); C and K are its parameters that are shared by all reaches. "Model for A" provides the stepwise linear regression used to predict the reach parameter A (with explanatory variables listed in the order they entered the regression). We indicate the proportion of HV variance ("% variance") explained by the generalized model (*i*) within the 99 reaches and (*ii*) in all reaches pooled ($n = 1980$ HVs).

Bates 1990; calculations were made with S-Plus 2000 (MathSoft™ Engineering and Education 1999)). These models are very attractive for providing simultaneous estimates of parameters that are constant between reaches (C and K, fixed parameters) and estimates of parameters that vary between reaches (A, random parameter). They provide model responses (HV–Re curves) that follow a similar functional form but depend on parameters that vary between groups (A). Fits were made using maximum-likelihood criteria. For each taxon, we chose the model providing the higher likelihood.

At the second step of the modeling procedure, we explained the overall level of HV curves in each reach (described by the reach parameter A estimated at the first step of the modeling procedure) as a function of average reach characteristics at mean discharge ($\mathrm{Fr_M}$, $\mathrm{Re_M}$, D/H_M, and W_M/H_M). Relationships were analyzed using stepwise (forward) linear regression (plots did not suggest nonlinearity). $\mathrm{Fr_M}$ and $\mathrm{Re_M}$ were log-transformed at this step to obtain distributions closer to normal.

We calculated the proportion of HV variance explained by the generalized models (*i*) within reaches (to reflect how the models explained the shape of HV–Re curves) and (*ii*) in all reaches (to reflect how the models explained HV at any stage in any reach).

Testing the robustness of the generalized models

We used an additional set of 14 New Zealand reaches with mean discharges that were outside the range of those used for developing the model (three reaches with Q_M between 0.05 and 0.09 $\mathrm{m^3 \cdot s^{-1}}$ and 11 reaches with Q_M between 57 and 552 $\mathrm{m^3 \cdot s^{-1}}$) to test whether our generalized models were robust outside their calibration range. We estimated HVs in these reaches using RHYHABSIM, and calculated the proportion of their variance explained by our generalized models.

Finally, we tested if our generalized models were transferable across different geological and hydrological contexts (for different species with comparable habitat-suitability curves). For this purpose, we ran RHYHABSIM to estimate HVs for French suitability curves in the 99 reaches with Q_M between 0.5 and 55 $\mathrm{m^3 \cdot s^{-1}}$ in New Zealand, and we calculated the proportion of their variance explained by the "French" generalized models of Lamouroux and Capra (2002).

Results

Modeling HV from average characteristics of stream reaches (generalized models)

The generalized models of HV (e.g., Fig. 3) for each taxon explained between 54% (for food production) and 80% (for inanga) of overall (all reaches pooled) variations in HV in all simulations (Table 1). HVs for six taxa were best fitted by model 1. For these taxa, modeled HV was maximum for Re = C/K (i.e., Re = 0.12 for rainbow trout, 0.25 for torrent fish, 0.28 for brown trout, 0.31 for food production, and 0.40 for longfin eel and *C. humeralis*; see Table 1). For the other taxa (redfin bully, upland bully, feeding inanga). HV decreased continuously with Re and was best fitted by model 2.

The generalized models also explained between 68% (longfin eel) and 88% (redfin bully) of HV variance within reaches (Table 1). Thus, the functional forms of models 1 and 2 were able to reflect the common shapes of HV changes with flow within reaches, and the hypothesis of common *C* and *K* values across reaches was sound (Fig. 3). For example, for brown trout, across the 52 reaches with Re values above 0.4 (i.e., reaches with a maximum HV within the modeling range), the maximum of the generalized HV curve at Re = 0.28 was between 86% and 100% (average 96%) of the maximum HV of the conventional curve.

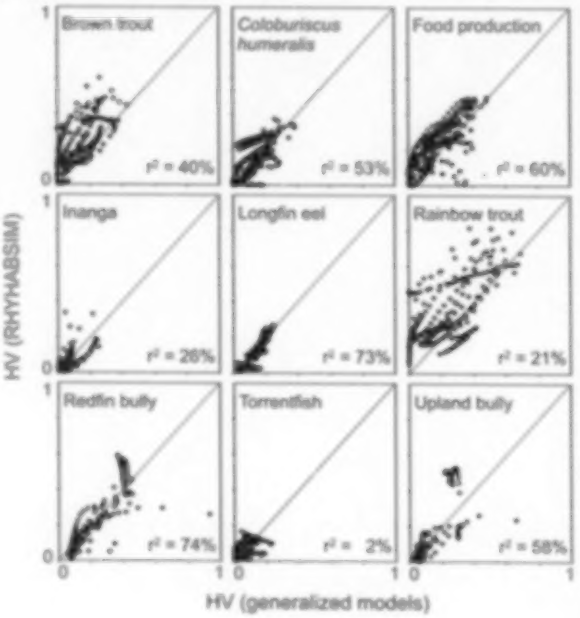
Equations for parameter *A* indicated that the overall level of HV in a reach varied differently across taxa (Table 1). The mean Fr for reaches (Fr_M) influenced *A* values for all taxa, indicating a strong impact of stream longitudinal morphology and hydraulics on HVs. Fr_M was the first variable to enter the regression for all taxa except the upland bully (which entered the regression just after W_M/H_M). Examination of partial correlations between Fr_M and *A* indicated that the nonbenthic species, brown trout, rainbow trout, and inanga, had higher HVs in reaches dominated by pools (low Fr_M). The remaining taxa were benthic and had higher HVs in reaches dominated by riffles. To a lesser extent, reaches with a high Re_M (high mean discharge per unit width) had high HVs for trout and inanga, whereas reaches with a low Re_M favored *C. humeralis*, longfin eels, upland bullies, and torrent fish, reflecting their preference for wide, shallow riffle habitats. Reaches with high D/H_M values had high HVs for five taxa, but D/H_M lowered HVs for torrent fish and upland bully. Large width to depth ratios favored the upland bully.

Testing the robustness of the generalized models

The models predicted between 53% and 74% of HV variance in stream reaches with mean discharges outside those in reaches used to formulate the models for five of the nine taxa (Fig. 4). Only 2% and 26% of HV variance was predicted for torrent fish and inanga, respectively. This was because there was little variation in HV for these two taxa in the 14 reaches, with the generalized models predicting very low HVs as was calculated from survey results (Fig. 4). However, predictions were not very satisfactory for adult rainbow and brown trout, for which 21% and 40% of HV variation was explained, respectively.

The generalized model of Lamouroux and Capra (2002) correctly predicted the HVs in New Zealand rivers that were obtained with French suitability curves (e.g., Fig. 5). Predicted variances in HV were 82%, 72%, and 83% for barbel, stone loach, and minnow, respectively.

Fig. 4. Observed HVs (outputs of the RHYHABSIM software) versus predicted ones (using our generalized habitat models) for the 14 reaches smaller or larger than those used to build the generalized model (reaches are pooled for each taxon). The line of perfect agreement (*y* = *x*) is given (solid line), as well as the proportion of variance in HV predicted.



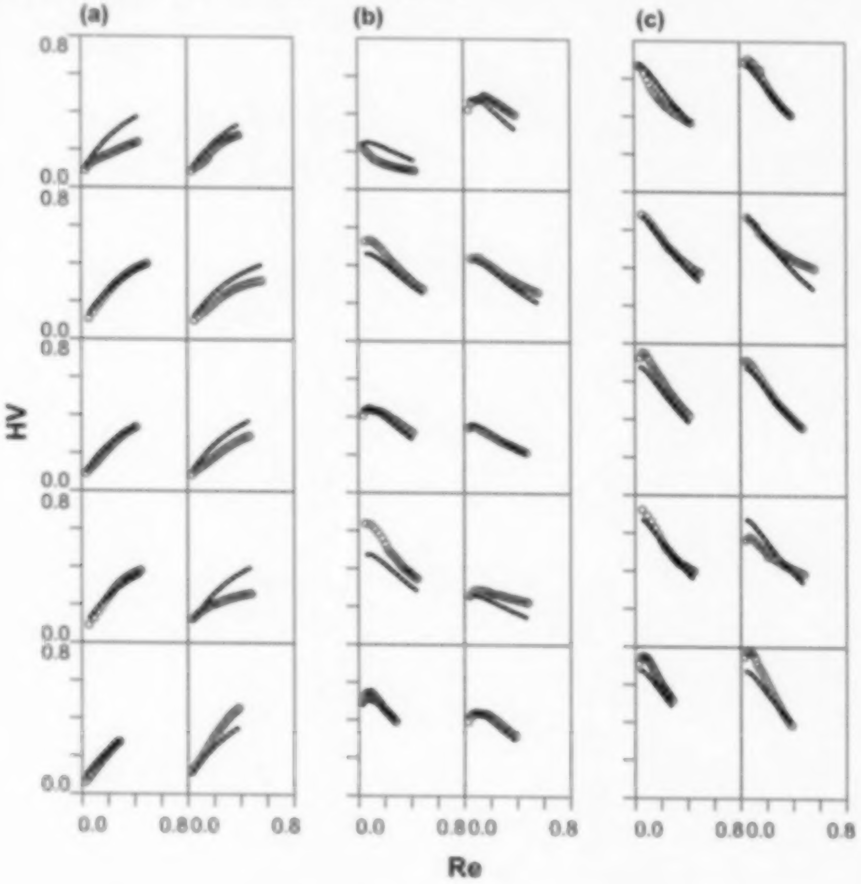
Discussion

As was found in France (Lamouroux and Capra 2002), HVs for taxa of New Zealand rivers were predictable from aggregated hydraulic data. Reach hydraulic geometry (mean depth–discharge and mean width–discharge relationships), average bed particle size, and mean natural annual discharge can be used to obtain reliable estimates of HVs in natural stream reaches. For all taxa involved in this study, these variables explained between 54% and 80% of HV variability. Changes in HVs within reaches and between reaches were both predicted by our models. Note that WUA in reaches can be estimated (from the average characteristics of reaches) by multiplying HV by the wetted surface area. Because the wetted surface area increases with discharge, maximum WUA will be reached for a discharge rate higher than the discharge rate that provides maximum HV.

The second major conclusion is that generalized habitat models such as ours are robust. Tests of the French models of Lamouroux and Capra (2002) in New Zealand rivers were very satisfactory, and most New Zealand models gave reasonable accuracy when applied to rivers larger or smaller than those used to calibrate them (with some loss of accuracy for some taxa). This suggests that the equations provided here are of potential use to model habitat quality anywhere in the world for taxa having comparable microhabitat preferences. However, we still recommend testing (and eventually adapting) the models in areas with geological properties and hydrological regimes contrasting with those found in New Zealand or Western Europe.

It is important to keep in mind that generalized habitat models are only models. They are based on conventional habitat models and therefore inherit most of their limitations (except their complexity). In particular, the interpretation of instream habitat models must be balanced by their limited biological validation (Lamouroux and Souchon 2002). Biological validation remains, in our view, the critical point in habitat modeling. Similarly, the generalized models should

Fig. 5. Observed HVs versus specific discharge (Re) curves (outputs of the RHYHABSIM software; ○) obtained using microhabitat suitability for three French taxa and predicted ones from average characteristics of reaches (solid lines show the "French" generalized habitat models of Lamouroux and Capra 2002) for 10 reaches selected at random among the 99 used. (*a*) Barbel (*Barbus barbus*). (*b*) Stone loach (*Barbatula barbatula*). (*c*) Minnow (*Phoxinus phoxinus*).



be used with much care outside their calibration range (e.g., in channelized or braided rivers).

Using our generalized models necessarily implies losses of information compared with conventional models such as PHABSIM (see the variance explained above). These losses must be balanced against the requirement for fieldwork and experience in conventional modeling. Conventional modeling involves three-dimensional topography of the bed and the water surface, measurement of georeferenced current velocities and water depths along a number of cross sections, calibration of a hydraulic model, and experience. By contrast, the input variables of our generalized models (mainly the hydraulic geometry relationships of reaches) can be obtained easily from a few field measurements or using regional models (Leopold et al. 1964; Jowett 1998; Lamouroux et al. 1998). A simple method of collecting this information consists of measuring about 100 depths and about 15 widths on a random grid covering the reach at two different discharge rates. Indeed, depth–discharge and width–discharge relationships for the reach can be confidently fitted to power laws using these measurements, for a wide range of discharge rates below bankfull (Leopold et al. 1964; Lamouroux et al. 1998).

Key physical variables driving HVs were found to be similar in New Zealand and France. The specific discharge governs changes in HV for all species within reaches. Fr for reaches at the mean natural discharge, which indicates the proportion of riffles in stream reaches, was generally the major variable governing the overall HV for the different reaches (reflected by our reach parameter A). This is consistent with the preference of the benthic fauna, such as many of the native New Zealand fish species and benthic invertebrates, for riffles (Jowett and Richardson 1995; Jowett 2000), and of the nonbenthic aquatic fauna for runs or pools (e.g., Jowett 2002).

We expect generalized habitat models to increase the panel of tools available for impact studies of river regulation because they are cost-efficient. They also suggest general, simple rules that can be used to improve flow management or to estimate regulation impacts over whole river networks. An example of such a rule is that a discharge of about $Q = 0.3 \cdot W$ (with Q in $m^3 \cdot s^{-1}$ and W in m) would provide optimal HVs for a number of freshwater taxa in New Zealand.

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List of symbols

Physical constants

v	water kinematic viscosity (considered throughout as equal to 10^{-6} $m^2 \cdot s^{-1}$)
g	gravitational acceleration (9.81 $m \cdot s^{-2}$)

Discharge-dependent reach characteristics

Q	discharge ($m^3 \cdot s^{-1}$)
H	reach-averaged water depth (m)
W	reach-averaged wetted width (m)
Fr	reach Froude number: $Q/(g^{0.5}\ H^{1.5}\ W)$
Re	reach Reynolds number: $Q/(v \cdot W)$. Throughout the paper, Re is multiplied by 10^{-6} to avoid working with high values. Practically, Re is a specific discharge estimated as Q/W

Discharge-independent reach characteristics

D	reach-averaged particle diameter (m)
Q_M	mean annual discharge ($m^3 \cdot s^{-1}$) under natural hydrology
H_M	H at Q_M (m)
W_M	W at Q_M (m)
Fr_M	Fr at Q_M
Re_M	Re at Q_M

Habitat values

HV	habitat value (ranging from 0 to 1)
WUA	weighted usable area, equal to HV × wetted surface area (m^2)

The impact of introduced round gobies (*Neogobius melanostomus*) on phosphorus cycling in central Lake Erie

David B. Bunnell, Timothy B. Johnson, and Carey T. Knight

Abstract: We used an individual-based bioenergetic model to simulate the phosphorus flux of the round goby (*Neogobius melanostomus*) population in central Lake Erie during 1995–2002. Estimates of round goby diet composition, growth rates, and population abundance were derived from field sampling. As an abundant introduced fish, we predicted that round gobies would influence phosphorus cycling both directly, through excretion, and indirectly, through consumption of dreissenid mussels, whose high mass-specific phosphorus excretion enhances recycling. In 1999, when age-1+ round gobies reached peak abundance near 350 million ($2.4\ \mathrm{kg \cdot ha^{-1}}$), annual phosphorus excretion was estimated at 7 t ($1.4 \times 10^{-3}\ \mathrm{mg\ P \cdot m^{-2} \cdot day^{-1}}$). From an ecosystem perspective, however, round gobies excreted only 0.4% of the phosphorus needed by the benthic community for primary production. Indirectly, round gobies consumed <0.2% of dreissenid population biomass, indicating that round gobies did not reduce nutrient availability by consuming dreissenids. Compared with previous studies that have revealed introduced species to influence phosphorus cycling, round gobies likely did not attain a sufficiently high biomass density to influence phosphorus cycling in Lake Erie.

Résumé : Un modèle bioénergétique basé sur l'individu nous a servi à simuler le flux de phosphore de la population de gobies à taches noires, *Neogobius melanostomus*, du centre du lac Érié en 1995–2002. Les estimations de la composition du régime alimentaire, du taux de croissance et de la densité de population du gobie à taches noires sont tirées d'échantillonnages de terrain. Comme c'est un poisson introduit abondant, nous avons prédit que les gobies à taches noires influenceraient le cycle du phosphore directement par leur excrétion et indirectement par leur consommation de moules dreissénidées dont l'excrétion de phosphore élevée en fonction de la masse favorise le recyclage. En 1999, au moment où les gobies à taches noires d'âge 1+ ont atteint leur abondance maximale de près de 350 millions d'individus ($2{,}4\ \mathrm{kg \cdot ha^{-1}}$), l'excrétion annuelle de phosphore a été estimée à 7 t ($1{,}4 \times 10^{-3}\ \mathrm{mg\ P \cdot m^{-2} \cdot jour^{-1}}$). À l'échelle de l'écosystème, cependant, les gobies à taches noires excrètent seulement 0,4 % du phosphore requis pour la production primaire de la communauté benthique. Indirectement, les gobies à taches noires consomment moins de 0,2 % de la biomasse de la population de dreissénidés, ce qui indique qu'ils ne réduisent pas la disponibilité des nutriments par leur consommation de dreissénidés. Contrairement à ce qui se passe dans des études antérieures où des espèces introduites peuvent influencer le recyclage du phosphore, les gobies à taches noires n'atteignent vraisemblablement pas des densités de biomasse nécessaires pour influencer le recyclage du phosphore dans le lac Érié.

[Traduit par la Rédaction]

Introduction

The role of zooplankters, benthic macroinvertebrates, and fish consumers as nutrient recyclers has gained increased attention (see review by Vanni 2002). These consumers can directly affect nutrient concentrations by consuming critical nutrients, sequestering some, and excreting the remainder as dissolved phosphorus (e.g., PO_4) for immediate uptake by primary producers (e.g., Lehman 1980; Braband et al. 1990). In addition, selective feeding by consumers and the resultant change in relative abundance of their prey can indirectly influence nutrient cycling should the prey species differ in rates or ratios of nutrient excretion (e.g., Shapiro and Wright 1984; Vanni et al. 1997). Overall, zooplankters and other invertebrates likely contribute more to nutrient recycling than fishes because these invertebrates are generally smaller and possess a greater population biomass and higher mass-specific excretion rate than fishes (e.g., Hudson et al. 1999).

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In some circumstances, however, fishes can have considerable impact on nutrient recycling (Vanni 2002). For example, fishes can consume prey in one habitat and excrete those nutrients in another, thereby providing new nutrients to a particular habitat of a lake (e.g., Braband et al. 1990; Schaus et al. 1997). In some cases, nutrient recycling by fishes can exceed the external nutrient input to the lake or reservoir (Braband et al. 1990; Schaus et al. 1997; Gido 2002). Successful introductions of fishes also may have considerable impact on nutrient recycling because they can attain high biomasses (e.g., Eck and Wells 1987) and can influence the composition of the food web (e.g., Brooks and Dodson 1965).

Despite the increasing frequency of fish introductions in the past century (see Rahel 2000) and their potential direct and indirect effects on lake nutrient cycling, relatively few studies have examined these effects. One example of a direct effect on phosphorus cycling is trout introduced into fishless oligotrophic Sierra Nevada lakes in North America. Their phosphorus excretion was estimated to be equivalent to the atmospheric deposition of phosphorus to the lakes (Schindler et al. 2001). Another study estimated phosphorus excretion and egestion by alewife (*Alosa pseudoharengus*) in Lake Michigan during the 1970s to be comparable with the phosphorus cycling by the zooplankton community (Kraft 1993). The introduction of northern pike (*Esox lucius*) into an experimental Canadian lake provided an example of fish indirectly influencing nutrient cycling. The pike eliminated all of the minnows, which in turn increased the biomass of large-bodied cladocerans and resulted in higher concentrations of dissolved phosphorus and nitrogen in the water column (Elser et al. 2000). Hence, introduced fishes can both directly and indirectly influence nutrient cycling in lake ecosystems.

In addition to the alewife, the Laurentian Great Lakes have experienced several fish introductions in the past century that may have influenced nutrient cycling. In this paper, we sought to determine whether the round goby (*Neogobius melanostomus*) population has influenced phosphorus recycling in Lake Erie since its introduction in 1993. This benthic molluscivore underwent an exponential increase in abundance over the next 5 or so years, attaining extremely high densities (40–100 fish·m^{-2} (Vanderploeg et al. 2002; Steinhart et al. 2004)) in preferred rocky and cobble substrates (Jude and DeBoe 1996). Our field sampling in the central basin of Lake Erie revealed round gobies to rank among the top three species, in terms of biomass, during August bottom trawl surveys of 1997–2000. Ironically, another introduced species, the zebra mussel (*Dreissena polymorpha*), may have facilitated the success of the round goby (Vanderploeg et al. 2002). Zebra mussels are the primary prey item of larger (>70 mm) round gobies (Ray and Corkum 1997; French and Jude 2001) and enhance the habitat for other benthic macroinvertebrates (e.g., amphipods and isopods) that are the primary prey item of smaller round gobies (French and Jude 2001).

Unlike round gobies, the effects of zebra mussels on nutrient cycling in the Great Lakes have been fairly well described. Owing to their high biomass and filtering capacity, zebra mussels can have dramatic impacts on nutrients (Heath et al. 1995; Mellina et al. 1995; Arnott and Vanni 1996), bacteria (Cotner et al. 1995), and phytoplankton (Madenjian 1995). Zebra mussel excretion of dissolved phosphorus and nitrogen can be a considerable source of nutrient regeneration in the Great Lakes (Heath et al. 1995; Arnott and Vanni 1996). In recent years, quagga mussels (*Dreissena bugensis*), another introduced species and a congener to zebra mussel, also have become widespread and dominate much of the dreissenid community in Lake Erie (Vanderploeg et al. 2002). Because we do not yet know the effect of quagga mussels on nutrient recycling, we will assume that their effects are the same as those of zebra mussels and will collectively refer to these exotic mussels as dreissenids.

Because round gobies have become abundant in Lake Erie and consume dreissenids, which are important nutrient recyclers, we hypothesized that round gobies may be directly and indirectly influencing nutrient cycling in Lake Erie. Using a mass balance bioenergetic modeling approach, we calculated the phosphorus excreted by round gobies (direct effect) in the Lake Erie central basin, where we have annual estimates of round goby abundance, diet, growth, and mortality. As one of the few fishes that exploit dreissenid mussels as prey, round gobies can provide a "new" source of phosphorus to benthic primary producers by releasing phosphorus bound in mussel tissue. At the same time, however, their consumption of dreissenid mussels also may indirectly influence phosphorus recycling. We used model results to evaluate whether the newly available phosphorus excreted by gobies is offset by the phosphorus that would have been excreted by those dreissenid mussels had they not been consumed (herein referred to as potential excretion). Even more simply, we compared the modeled annual consumption of dreissenids by round gobies with estimates of dreissenid standing stock biomass to determine whether round gobies may be imposing a measurable top-down influence on dreissenids. In total, providing annual estimates of round goby excretion and consumption of dreissenid mussels will contribute to a greater understanding of the role of exotic species in altering nutrient dynamics in central Lake Erie.

Methods

Our general approach was to use the mass balance approach of Kraft (1992) to estimate consumption, excretion, and accumulation of phosphorus by individual round gobies in the central basin of Lake Erie. To estimate this phosphorus flux, we incorporated annual field-based estimates of round goby growth, diet, population abundance, and mortality into an individual-based model, which allowed us to monitor the daily consumption and excretion of phosphorus by individual fish of varying size within a yearly cohort. Conventional bioenergetic model simulations use one fish of average size to represent the cohort. Because many of the equations in the bioenergetic model are size dependent (and many are nonlinear), we used an individual-based model to preserve the observed variation in size among individuals, which in turn should provide more realistic estimates of the bioenergetics of the population. We extrapolated the flux of the simulated individuals in the individual-based model to the number of individuals estimated in the population to calculate the annual rates of phosphorus flux by the population.

Study site

Lake Erie, the shallowest and most productive of the Laurentian Great Lakes, has three distinct basins that vary in depth and productivity. We focused on the central basin of Lake Erie, which is intermediate in depth (mean = ~18 m) and productivity to the western basin (shallow, most productive) and eastern basin (deep, least productive). Unlike the western basin, the central basin is deep enough to stratify during summer. Nearly every year in the central basin, oxygen concentrations in the hypolimnion become hypoxic (<2 mg·L^{-1}) during late summer (Bertram 1993). Since 1995, concentrations of total phosphorus have increased in the central basin (Charlton and Milne 2004), despite relatively constant phosphorus loadings (D. Dolan, Department of Mathematics, University of Wisconsin-Green Bay, Green Bay, WI 54311 USA, unpublished data), which has led to increasingly hypoxic conditions. If internal phosphorus recycling is responsible for these higher phosphorus concentrations, then it is possible that the introduction and success of round gobies may be influencing phosphorus dynamics in the central basin.

Population sampling

For annual estimates of individual round goby growth rates, population abundance, and diet composition, round gobies were sampled with a bottom trawl (10.4-m head rope, 1.3-m vertical opening, 15.2-m roller sweep, and 6-mm cod-end liner) that effectively sampled a variety of substrates (silt, mud, shale, rocks, and boulders). Sampling occurred at least four times annually between May and October 1995–2002 (gobies were first sampled in Lake Erie in 1994). For each month of sampling, trawls sampled two to nine standardized transects in the central basin. Along each transect, replicate trawls were completed at each of four depth strata: 5 m (5–9.99 m), 10 m (10–14.99 m), 15 m (15–19.99 m), and 20 m (≥20 m). Trawls at sites <5 m depth were not possible. The duration of the trawls ranged from 2 to 15 min (mean = 9.6 min), and the distance covered by the trawl was calculated by multiplying the minutes trawled by the trawling speed of 2.5 kn. Multiplying the distance covered by the width of the trawl estimated the area swept for each trawl. For each trawl, at least 30 round gobies were randomly selected for measurement of total length (TL) (nearest millimetre) and at least five fish per estimated size-class (<40 mm, between 40 and 100 mm, and >100 mm) were frozen from each site for later measures of age, diet, and sex. Round goby ages were estimated from scales; aging by scales was validated by otoliths (C.T. Knight, unpublished data). Age-0 round gobies were rarely captured in the trawls, as they had not yet grown to a size large enough to be sampled. Hence, all references to round gobies in this paper will refer only to those at least 1 year of age.

All diet items were enumerated and identified to genus. Fish prey were measured in length and their wet weight was estimated from length–weight regressions (Ohio Division of Wildlife 2003). For all other prey items, a mean wet weight was applied, which was calculated from a compilation of lengths and weights from prey items collected between 1990 and 1996 in Lake Erie. We categorized diet items into one of six categories: dreissenid mussels, other mollusks (75% of which were other bivalves), Chironomidae, other benthos (45% of which were Amphipod and 22% of which were Trichoptera), zooplankton (58% ostracods, 17% copepods, and 12% *Bythotrephes longimanus*), and fish (27% clupeids and 22% round gobies). We used wet mass to determine the proportion of each diet category as a function of habitat, season, year, and size. Habitat was defined as either nearshore (between 5 and 15 m depth) or offshore (≥15 m depth). We chose 15 m as a cutoff because the percentage of non-mud habitat differed considerably between nearshore (72%) and offshore (26%) (Haltuch and Berkman 1998), which may affect prey assemblages. Seasons for the diet analyses were partitioned as spring (May–June), summer (July–August), and autumn (September–October); winter diets were estimated as the interpolation between the diet proportions of autumn and spring. We divided the diets of round gobies into three size-classes, which approximated age-classes: <70 mm TL (through autumn of age-1 fish), 70–110 mm TL (between autumn of age-1 and age-2), and >110 mm TL (age-3+ fish). When fewer than three stomachs were available for a given season, size, habitat, and year combination (occurred for 7% of the inshore and 39% of the offshore combinations), we substituted the average of all other years with similar seasons, sizes, and habitats.

Round goby density was estimated as a function of depth strata, sex, age, and year. To determine the length distribution in each trawl, we multiplied the proportion of measured fish in each centimetre length bin by the total catch. To determine the sex ratio in each catch, we calculated a sex–length key by pooling all fish that were measured and identified to sex across all years and calculating the sex ratio for each centimetre length bin. Similarly, to determine a sex-specific age–length key, we pooled all fish that were identified to sex and aged in all years. With these probabilities in hand, we calculated the density of round gobies in each trawl as a function of age and sex. To estimate density of round gobies at each depth strata, we calculated the mean density, with 90% confidence limits, across all sites and months within each depth strata. For each depth strata, we multiplied the mean density (and confidence limits) by its total area in the central basin (estimate by Haltuch and Berkman 1998) to estimate population abundance. In our models, we assumed that this abundance represented the number of fish on 1 June of each year.

To estimate total annual mortality, we used a catch-curve analysis. We calculated the total abundance of each age-class for each sex in each year. The negative slope of the $\log_e$-transformed data (without the age-0 or age-4 gobies, as they were so rare that including them biased the slope) estimated z.

Individual-based model

We used an individual-based model to simulate the phosphorus flux by individuals from representative "groups" of the round goby population. For each year, 1995–2002, we ran two replicate individual-based model simulations for each group of round gobies, which comprised a particular sex (male or female), age (1, 2, 3, or 4), and habitat (nearshore or offshore). Because age-4 females were never sampled in either habitat, that group of simulations was not run. Within the individual-based model, we monitored the grams of phosphorus that each individual had consumed, mobilized to

Table 1. Grand mean mass and SD of round gobies in the central basin of Lake Erie at initiation of each model run (i.e., 1 January) across simulated years 1995–2002.

	Males		Females	
Age (years)	Grand mean mass (g) across years	SD of grand mean across years	Grand mean mass (g) across years	SD of grand mean across years
1	2.97	0.0	2.97	0.0
2	11.70	1.06	9.45	0.68
3	33.71	2.32	24.21	2.85
4	67.16	2.70	—	—

Note: No simulations were run for age-4 females.

new tissue (i.e., growth), and excreted. At the initiation of each model run, 1 January each year, 1000 fish were drawn from a normal distribution of masses, where the mean and standard deviation were estimated from aged fish collected in the field (Table 1). Each day, each fish consumed a diet whose proportional contents were determined by the field-based observations (a function of year, habitat, season, and fish size) and grew according to a bioenergetics subroutine (see next section). The amount of prey consumed was set by the *p* value, the proportion of maximum consumption. For all groups of fish, we used the *p* value that allowed fish to grow at the same rate in the simulation as fish sampled from the field. The daily probability of survival equaled $s^{1/365}$, where *s* was the sex-specific annual survival rate.

As batch spawners, mature gobies were assumed to spawn four times each year. We assumed spawning to begin when temperatures warmed to 12 °C (Moiseyeva and Rudenko 1996). Assuming a 21-day spawning interval (Kulikova 1985; L. Corkum, Department of Biological Sciences, University of Windsor, Windsor, ON N9B 3P4, Canada, personal communication), gobies were modeled to spawn 20 May, 17 June, 15 July, and 5 August each year (using the average daily temperature over the years 1994–2002). Females were assumed to mature at age 2 and males at age 3. Because batch spawners do not expel all gonadal tissue each spawning event (i.e., structural and immature tissue), we assumed that 75% of the gonadal tissue was released each event. With a gonadal mass estimated at 1.35% of somatic mass for mature males (Belanger 2002) and 9.1% for mature females (MacInnis and Corkum 2000; Belanger 2002), males and females expelled 1% and 6.8%, respectively, of body mass at the end of each spawning day.

Mass balance approach to estimate phosphorus flux

With the mass balance, we assumed that phosphorus consumed (C_P) was used to build new tissue (i.e., somatic or gonadal growth (G_P)), egested as organic fecal waste, or excreted (U_P) as soluble inorganic waste. Because egestion was assumed to be 28% of phosphorus consumed (Nakashima and Leggett 1980), phosphorus excretion was estimated as (Kraft 1992)

$$(1) \qquad U_P = (0.72C_P) - G_P$$

To estimate C_P and G_P, we required wet mass estimates of round goby consumption and growth as well as estimates of the phosphorus concentrations of round gobies and their prey. Daily estimates of C_P and G_P were generated from the bioenergetics subroutine of the individual-based model, which included parameters derived for round gobies (Lee 2003) and equations from the Wisconsin bioenergetics model (Hanson et al. 1997). The bioenergetics model balances energy consumed with energy used in metabolism, growth, excretion, and egestion, given a known growth rate, temperature regime, diet composition, and energy density of predator and prey. As previously described, growth and diet composition were estimated from round gobies sampled in central Lake Erie. Daily temperature was represented as the average water temperature collected from four municipal water intake sites across the central basin: (*i*) Cleveland, Ohio (8046 m offshore, 13.0 m depth), (*ii*) Painesville, Ohio (366 m offshore, 3.7 m depth), (*iii*) Wheatley, Ontario (426 m offshore, 6.4 m depth), and (*iv*) Elgin, Ontario (1250 m offshore, 9.8 m depth). Estimates of energy density for the six prey categories were compiled from the literature (Table 2). In categories with multiple taxa, we used their average proportional abundance of the primary taxa to weight the values (Table 2). Energy density of round gobies in Lake Erie was modeled to increase with size (G.W. Kim, Department of Evolution, Ecology, and Organismal Biology, Ohio State University, 1314 Kinnear Road, Columbus, OH 43212, USA, and T.B. Johnson, unpublished data) according to

$$(2) \qquad \text{J·g wet mass}^{-1} = 2942.86 \times \text{mass}^{0.08646}$$

Grams of phosphorus consumed (C_P) were estimated by multiplying daily consumption (grams) of each prey item by its phosphorus concentration that was estimated from the literature (Table 2). Similarly, grams of phosphorus into growth (G_P) were estimated by multiplying daily growth of the round goby by its phosphorus concentration (Table 2), which we estimated from 30 round gobies (two or three fish per centimetre size-class, sizes ranged from 3 to 16 cm TL) sampled from the central basin of Lake Erie during May–June 2002.

To estimate the phosphorus concentrations of round gobies, we removed the contents of the stomach and intestines from each fish and the remaining tissue was dried at 60 °C until a constant mass (nearest 0.001 g) was reached. The tissue was then homogenized with either a mortar and pestle or a Retsch ZM 100 tissue grinder (Newtown, Pennsylvania) and then dried again for 3 days at 60 °C. For each of three replicate samples (0.3–1.0 mg each) per fish, phosphorus was converted to soluble reactive phosphorus with a hydrochloric acid digestion (Stainton et al. 1974) and then estimated with a Lachat FIA+ QuikChem 800 series autoanalyzer (Loveland, Colorado) (samples run by M. Vanni, Department of Zoology, Miami University, Oxford, OH 45056, USA). Because phosphorus concentrations did not increase with

Table 2. Literature-compiled estimates of energy density and phosphorus concentrations for the six prey categories of round gobies in the central basin of Lake Erie.

Category	Energy density ($J\cdot g$ wet mass^{-1})	Reference(s)	Phosphorus concentration ($g\ P\cdot g$ wet mass^{-1})	Reference(s)
Dreissenid mussels	2427[a]	Schneider 1992	0.0015[a]	Arnott and Vanni 1996
Other mollusks	2427[b]	Cummins and Wuycheck 1971	0.0015[b]	Arnott and Vanni 1996
Chironomidae	2745	Cummins and Wuycheck 1971	0.0019	Penczak 1985
Other benthos	3969[c]	Cummins and Wuycheck 1971	0.0019[c]	Penczak 1985
Zooplankton	3235[d]	Cummins and Wuycheck 1971; Vijverberg and Frank 1976	0.001[e]	Cummins and Wuycheck 1971; Vijverberg and Frank 1976
Age-0 fish	2500	Post 1990; T.B. Johnson and G.W. Kim, unpublished data	0.005	Kraft 1992; this study

[a] Zebra mussel soft tissue only, as shells are not digested by round gobies. Assumed that quagga and zebra mussels are equal.
[b] Assumed equal to dreissenid mussels.
[c] Weighted average of Amphipoda and Trichoptera.
[d] Weighted average of Ostracoda (assuming average crustacean), cyclopoids, and *Bythotrephes longimanus* (assuming average Cladocera).
[e] Weighted average of Ostracoda (assuming $p = 0.1\%$ of dry mass), cyclopoids, and *B. longimanus* (assuming average Cladocera).

round goby size ($F_{[1,28]} = 3.17$, $r^2 = 0.10$, $p = 0.09$), we used a mean phosphorus concentration of 0.00585 g P·g wet tissue^{-1} for all round gobies.

We compiled the 28 different individual-based model results each year to calculate the round goby population contribution to phosphorus cycling. For each day in each model run, we added the phosphorus consumed, mobilized into growth of new tissue, and excreted across the surviving fish. When fish were losing mass (i.e., growth was negative), we set phosphorus growth to zero so as not to overestimate phosphorus excretion (Kraft 1992). Within each group of ages, sexes, and habitats, we then calculated the mean daily phosphorus flux across the two replicates. To extrapolate the individuals in the individual-based model to the population, we used an extrapolation coefficient, which equaled the number of surviving fish on 1 June in the individual-based model divided by our field-based estimate of round goby abundance. For each day of the model, we then divided the relevant model output (e.g., number of individuals, phosphorus excretion) by the extrapolation coefficient to extrapolate the dynamics of the modeled individuals to the central basin population.

Results

Population sampling

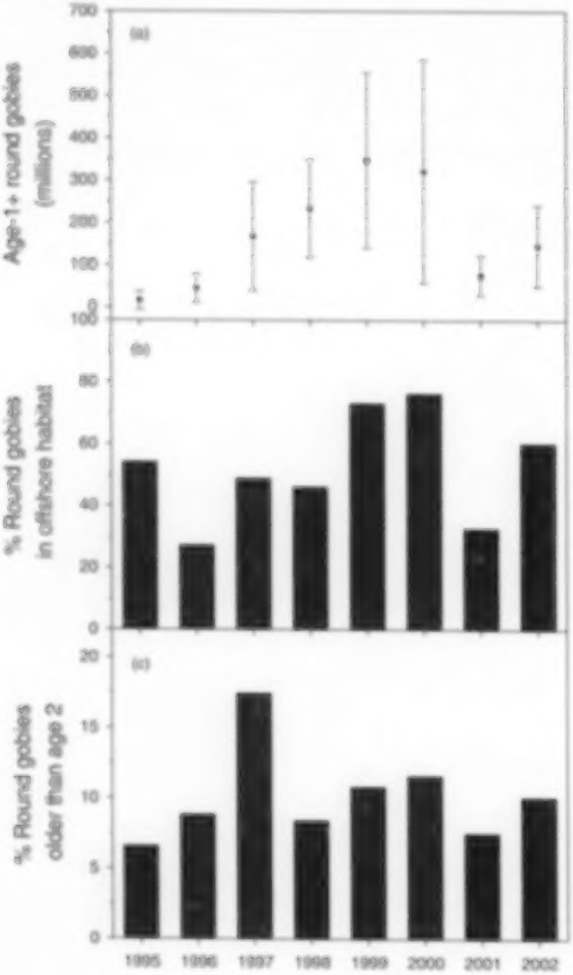
Because age-0 round gobies had not recruited to the bottom trawl, our population estimates include only age-1+ fishes. Estimates of mean annual abundance of age-1+ round gobies in the central basin of Lake Erie varied between 1995 and 2002. After round gobies were first sampled in 1994 (C.T. Knight, unpublished data), the population increased considerably until it reached a peak in 1999 of nearly 350 million fish (Fig. 1*a*). Across all years, mean densities of round gobies were greater in the nearshore habitat (mean = 307 individuals·ha^{-1}, range = 34–583) than in the offshore habitat (mean = 81 individuals·ha^{-1}, range = 7–201). However, round gobies in the offshore habitat comprised at least 46% of the total population size in six of the eight years (Fig. 1*b*) because of the larger area of offshore habitat. With regard to sizes of gobies sampled during the survey, the average TL of the more than 32 000 individuals measured (range = 1798–6279 fish across years) equaled 75 mm. The majority of individuals were between 30 and 79 mm TL in each year (range = 57.9%–69.0% of measured fish across years, mean = 63.4%), and <1% of individuals were <30 mm TL. The size distributions of gobies in each trawl were used to assign putative ages and sexes to the gobies for the population model. With respect to age-classes, the vast majority of round gobies were predicted to be age 1, as only 7.5%–17% of the age-1+ population was age 2 or older (Fig. 1*c*). This was a result of the length–age key that found at least 74% of round gobies between 30 and 79 mm TL to be age 1.

With regard to sex, males were estimated to be slightly more than 50% of the population in each year (range 50.3%–58.5%). This was a result of the sex–length key that found a high bias towards males at larger sizes. Females slightly dominated the sex ratio among fishes smaller than 80 mm TL (mean = 54.3% of the population). Males, however, predominated among fishes of larger sizes: 57% of fish 80–89 mm, 63% of fish 90–99 mm, 79% of fish 100–119 mm, more than 90% of fish 120–159 mm, and 100% of fish larger than 160 mm. Annual survival rates derived from catch-curve analyses also revealed differences between males and females. From 1995 to 2002, average annual survival for males was 58% (range = 35%–70%) compared with 19% for females (range = 14%–23%).

Individual-based population simulation of phosphorus flux

Using the population densities, diets, and rates of growth and survival derived from field sampling, we simulated individual round gobies (ages 1–4 of males and ages 1–3 of females in both nearshore and offshore habitat) during 1995–2002 to determine population rates of consumption and excretion. This model did not include age-0 round gobies from when they were hatched (May–August) until 31 December because our field samples did not collect age-0 round gobies. The interannual growth pattern of round gobies exhibited a general trend across years. Using 1998 as an example, all ages and sexes of simulated round gobies lost weight during winter (December–April) and began gaining weight in May (Fig. 2). The largest weight gains, however, occurred during autumn (October–November). Given

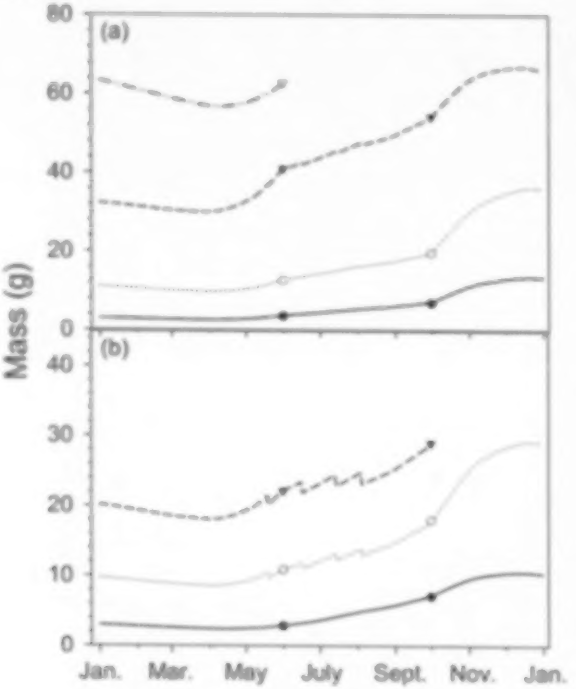
Fig. 1. Interannual population dynamics of age-1+ round gobies in the central basin of Lake Erie estimated from bottom trawls during May–October of each year. (*a*) The 90% confidence intervals around the annual mean abundance; (*b*) percentage of round gobies that were estimated to occur in the offshore habitat (i.e., ≥15 m depth) in each year; (*c*) percentage of round gobies (includes ages 1–4) that were estimated to be older than age 2 in each year.



that mean sizes of round gobies sampled 1 June and 1 October in the field were used to calibrate the growth rates of round gobies in the model, the growth trajectory of simulated gobies passed through the mean sizes of field-sampled gobies on those dates, as expected (Fig. 2).

Estimated annual consumption by age-1+ round gobies was influenced largely by their annual abundance. Consumption of prey peaked at nearly 25 000 t in 1999 (Fig. 3) (range of 9981 – 39 795 using 90% confidence interval from mean population abundance). Dreissenid mussels dominated the diet in each year, ranging from 40.8% in 1997 to 57.2% of the diet in 1998. The second most abundant prey item varied across years: chironomids (1995, 1998, 2000, and 2002), mollusks other than dreissenids (1997 and 1999), and zooplankton (1996 and 2001).

Fig. 2. Comparison of mean masses estimated from round gobies in the Lake Erie central basin during June and October 1998 (symbols) with daily mass of round gobies in the model simulation (lines). Open triangles are age-4 fish, solid triangles are age-3 fish, open circles are age-2 fish, and solid circles are age-1 fish. (*a*) Males; (*b*) females. Note the difference in the scales on the *y* axes.



Interannual variation in round goby population abundance drove interannual changes in the contribution of round gobies to phosphorus cycling in the lake. The average amount of phosphorus bound in age-1+ round goby population biomass was as low as 0.8 t in 1995 and peaked at 23.9 t in 1999, when mean round goby abundance was highest. Annual tonnes of phosphorus consumed by age-1+ round gobies reached a maximum of 39 t (15.7–62.3 t, 90% confidence interval) in 1999 (Fig. 4*a*). Tonnes of phosphorus mobilized into new round goby tissue (i.e., growth) were slightly higher in 2000 (mean = 24.5 $t \cdot year^{-1}$, 4.7– 44.3 t, 90% confidence interval) than in 1999 (mean = 24.4 $t \cdot year^{-1}$, 9.8–39.1 t, 90% confidence interval) (Fig. 4*a*). Excretion of dissolved phosphorus was highest in 1999, at 7.3 $t \cdot year^{-1}$ (3.0 –11.7 t, 90% confidence interval) (Fig. 4*a*). Finally, excretion that could be attributed to round goby consumption of dreissenid mussels (i.e., newly available phosphorus) also was highest in 1999 (mean = 3.5 $t \cdot year^{-1}$, 1.5– 4.5 t, 90% confidence interval) (Fig. 4*b*).

Within a given year, phosphate excretion varied seasonally. Because consumption rates (i.e., *p* value) in the model were changed on 1 June and 1 October, abrupt changes in excretion occurred on those dates. Although these sudden changes likely are not representative of nature, the model

Fig. 3. Estimated mean annual consumption of six different prey items by the age-1+ round goby population in the Lake Erie central basin during 1995–2002.

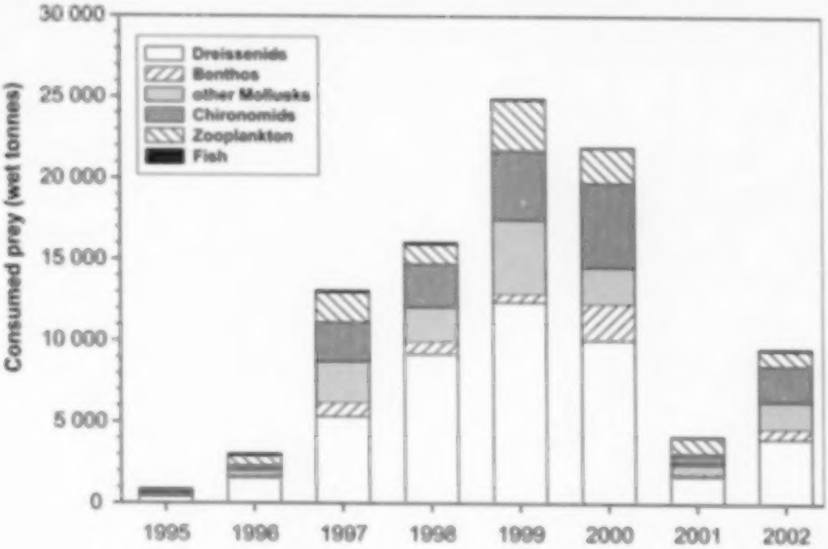
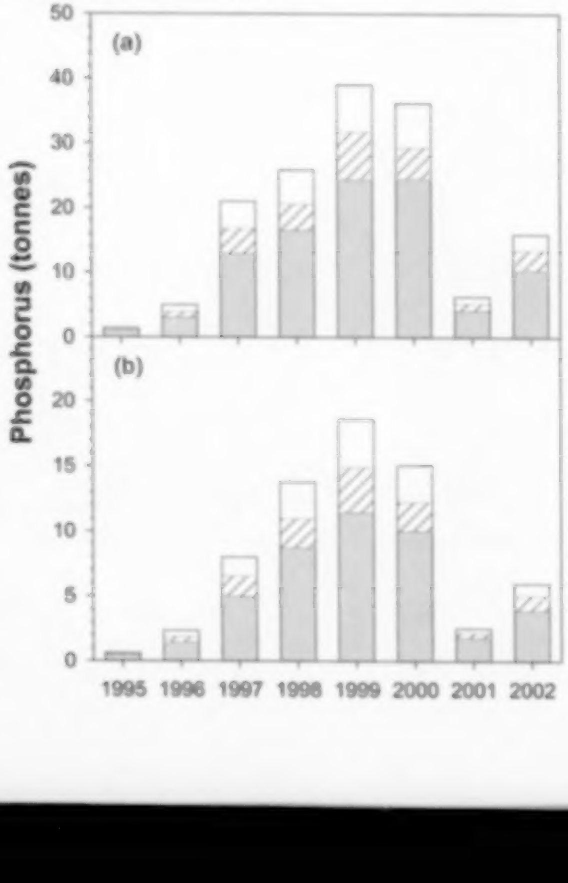


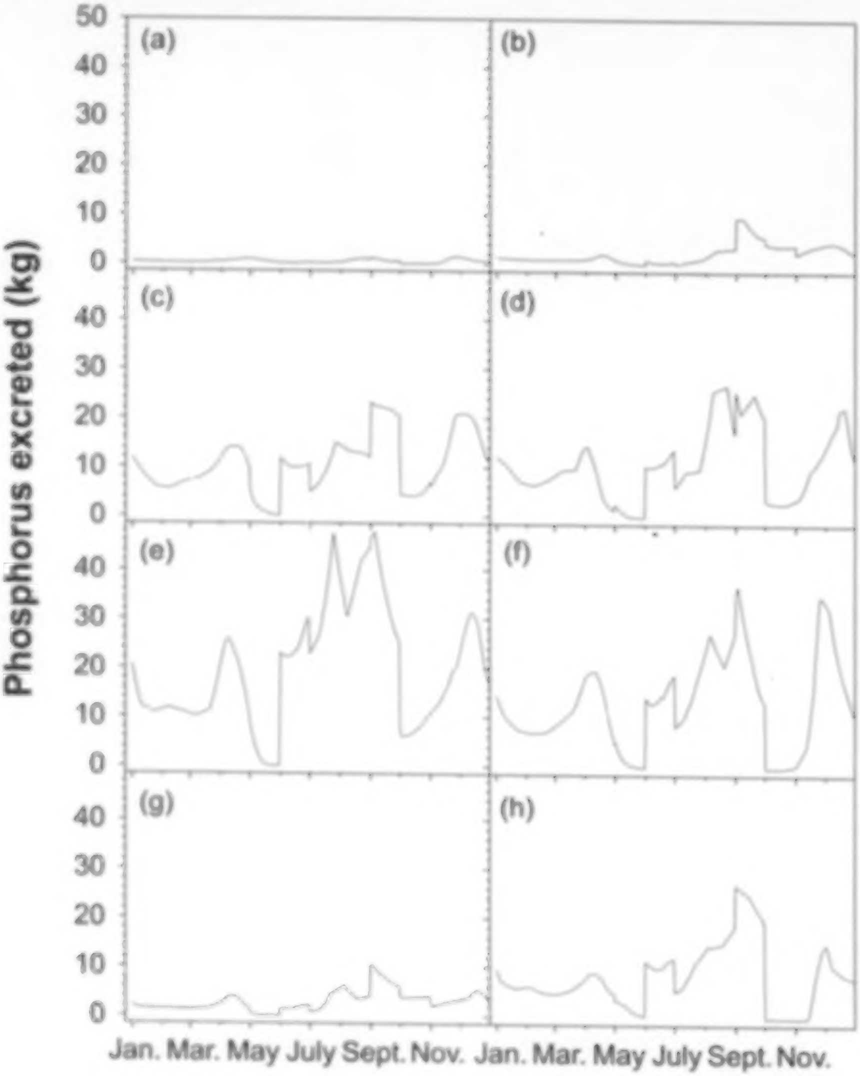
Fig. 4. Annual phosphorus dynamics of age-1+ round gobies in the Lake Erie central basin. The shaded bars depict phosphorus mobilized into new tissue, the hatched bars depict phosphorus excreted, and the open bars depict phosphorus egested. The height of each bar represents the total phosphorus consumed. (*a*) Phosphorus consumed from all prey items; (*b*) phosphorus that can be attributed to consumption of dreissenid mussels. Note the different scales on the *y* axes.



can reveal relative changes in excretion through the year. Most of the phosphorus was excreted between 1 June and 1 October, when warm water temperatures and moderate growth rates resulted in high consumption and residual phosphorus for excretion (Fig. 5). Another pulse of excretion occurred around December in most years, when end-of-the-year growth had begun to taper off but temperatures remained sufficiently warm for consumption to continue. During the coldest months of January and February, however, consumption and excretion remained very low. During April, another pulse of phosphorus was excreted by the round gobies as consumption increased with the warming spring temperatures.

To determine the indirect effects of round gobies on phosphorus regeneration, we first estimated how much potential dreissenid excretion was lost because of round goby consumption of dreissenid mussels. To estimate potential excretion by dreissenids, we assumed a phosphorus excretion rate of 3.16×10^{-5} g·g dry mass^{-1}·h^{-1} (Arnott and Vanni 1996; estimated for zebra mussels in Lake Erie during summer), an 85% water composition for soft tissue (Schneider 1992), and that dreissenids were open (i.e., filtering and excreting) for 19 h·day^{-1} (Walz 1978). Because we know zebra mussel excretion estimates for the summer months only, we estimated potential excretion from June through September of each year. The mean potential excretion by dreissenid mussels ranged from 142 g P·day^{-1} when round gobies were not abundant in 1995 to more than 4249 g P·day^{-1} in 1999 when round gobies reached their peak abundance (Table 3). In each year, however, the new phosphorus excreted by round gobies, owing to their dreissenid mussel consumption, exceeded the phosphorus that would have been excreted by those mussels had they not been consumed (Table 3; Fig. 6). Taking into account the lost potential excretion from consumed dreissenids reduced the new phosphorus available to primary producers by an average of 60%.

Fig. 5. Dissolved inorganic phosphorus excreted by the age-1+ round goby population in the Lake Erie central basin as a function of day of year. (*a*–*h*) Years 1995–2002, respectively.



Finally, we compared the estimated consumption of dreissenid biomass with the standing stock biomass of dreissenids to evaluate whether round gobies could be indirectly modifying phosphorus recycling by influencing the biomass of dreissenids in the central basin. Because we could not find a time series of dreissenid biomass from 1995 to 2002, we used a mean dreissenid biomass of 878 $g \cdot m^{-2}$ (wet mass with shell), which was estimated across 19 sites in the central basin during 1998 (Jarvis et al. 2000). Assuming that 57% of the total wet mass was tissue (Johannsson et al. 2000; i.e., digestible), we approximated a basin-wide biomass abundance of 7.5×10^6 t of dreissenid tissue. In 1998, when round gobies were near their peak in abundance, our model estimated that 9171 t of dreissenid tissue was consumed, which translated to just over 0.1% of dreissenid standing stock biomass.

Discussion

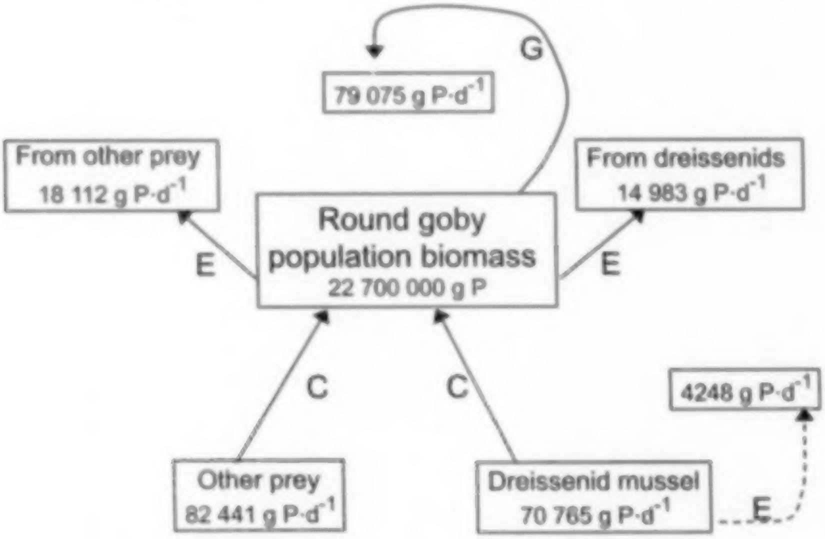
Since round gobies became established in Lake Erie, they have attained population abundance estimates as high as 350 million (age 1 and older) in the central basin. Phosphorus cycling by the population followed the general trend of the population abundance: increasing consumption, accumulation, and excretion of phosphorus from 1995 through 1999 and then a subsequent reduction in 2000–2002. At their peak in 1999, round gobies excreted more than 7 t of phosphorus. Because dreissenid mussels predominated their diets, most

Table 3. Annual indirect effect of round goby consumption of dreissenid mussels on available phosphorus to primary producers during June–September in the central basin of Lake Erie.

Year	Wet tissue of dreissenids consumed ($t\cdot day^{-1}$)	Wet phosphorus of dreissenids consumed ($g\cdot day^{-1}$)	Phosphorus excreted by round gobies from dreissenids ($g\cdot day^{-1}$)	Potential excretion by dreissenids that was consumed ($g\cdot day^{-1}$)	Net gain of new phosphorus ($g\cdot day^{-1}$)
1995	2	2 365	181	142	39
1996	6	9 370	1 208	563	645
1997	15	22 957	4 343	1378	2 965
1998	33	50 019	9 045	3003	6 042
1999	47	70 765	14 983	4249	10 734
2000	35	51 911	8 083	3117	4 966
2001	6	8 789	1 239	528	711
2002	12	17 286	5 052	1038	4 014

Note: Net gain of new phosphorus equals phosphorus excreted by round gobies from their consumption of dreissenid mussels minus the potential phosphorus excretion by dreissenid mussels that were consumed.

Fig. 6. Phosphorus dynamics associated with the age-1+ round goby population in the central basin of Lake Erie during June–September 1999, the year in which round gobies were most abundant. Solid arrows with their respective labels (G, growth; E, excretion; C, consumption) represent phosphorus rates from round gobies. The broken arrow from the box that represents consumed dreissenid mussels indicates the predicted phosphorus that would have been excreted by those dreissenids had they not been consumed by round gobies (i.e., potential excretion).



of the phosphorus excreted by round gobies can be considered newly available to the benthic community, as relatively few native predators consume and excrete the phosphorus bound in dreissenid tissue. Indirectly, however, consumption of dreissenid mussels also will reduce phosphorus available to primary producers because dreissenids are one of the most important phosphorus recyclers in Lake Erie (Arnott and Vanni 1996). Taking into account the lost potential excretion by dreissenids that were consumed reduced our estimate of new phosphorus available to primary producers (via round goby consumption of dreissenids) by an average of 60% across all years. In addition, our model results suggested that consumption of dreissenids was not sufficient to reduce their basin-wide biomass: round gobies were estimated to consume only around 0.1% of the estimated dreissenid standing stock biomass in 1998.

Round goby excretion rates in comparison with fishes in other systems

Compared with previous studies that have estimated fish phosphorus cycling, excretion by the round goby population in central Lake Erie was low (Table 4). Fish excretion rates, standardized by surface area, have ranged from 0.01 to 5.46 mg $P\cdot m^{-2}\cdot day^{-1}$ in previous studies. During 1995–2002, our estimates of round goby excretion ranged only 0.03–1.35 µg $P\cdot m^{-2}\cdot day^{-1}$, at least an order of magnitude smaller than all other published studies. This result could be the result of a low mass-specific excretion rate by individual round

Table 4. Summary of phosphorus excretion rates estimated for populations or assemblages of fishes in various lakes and reservoirs sorted as a function of fish biomass.

Lake or reservoir	Taxonomic grouping	Area (ha)	Mean depth (m)	Biomass ($kg \cdot ha^{-1}$)	Excretion rate ($mg\ P \cdot m^{-2} \cdot day^{-1}$)	Mass-specific excretion rate ($\mu g\ P \cdot g^{-1} \cdot h^{-1}$)	Source
Lake Erie, central basin	Round goby (*Neogobius melanostomus*)[a]	1 506 321	18	2	0.0014[b]	0.0–1.0[b]	This study
Sierra Nevada lakes (unstocked)	Trout[a]	1–13		3–17	0.01		Schindler et al. 2001
Sierra Nevada lakes (stocked)	Trout[a]	3–23		14–21	0.02		Schindler et al. 2001
Lake Pend Orielle	Kokanee salmon (*Oncorhychus nerka*)[a]	35 300	164	16	0.02		Chipps and Bennett 2000
Lake Köyliönjärvi	Roach (*Rutilus rutilus*)	1250	3	38	0.86		Tarvainen et al. 2002
Lake Michigan	Alewife (*Alosa pseudoharengus*)[a]	5 780 000	85	69	0.57[c]		Kraft 1993
Lake Memphremagog, south basin	Young-of-the-year yellow perch (*Perca flavescens*)	4371	7	70	0.14–2.06		Kraft 1992
West Long Lake	All fish	4	10	139	0.44		Schindler et al. 1993
Lake Texoma	Benthivores	36 000	8	192	0.96		Gido 2002
Peter Lake	All fish	2	8	199	3.18		Schindler et al. 1993
Union Lake	Common carp (*Cyprinus carpio*)[a]			200	0.52–1.07	0.5–10.0	Lamarra 1975
Lake Finjasön	Roach (*Rutilus rutilus*), bream (*Abramis brama*)	1100	3	229	0.53		Persson 1997
Lake Gjersjoen	Roach (*Rutilus rutilus*)	270	23	300	1.2–2.9	2.5–7.7	Braband et al. 1990
Lago Paranoá, Riacho Fundo	Tilapias (*Oreochromis niloticus*, *Tilapia rendalli*)[a]	446		341–791	0.88–2.04	0.5–1.6	Starling et al. 2002
Acton Lake	Gizzard shad (*Dorosoma cepedianum*)	253		417	5.46	2.2–21.4	Schaus et al. 1997

[a] Introduced species.

[b] In 1999, the year in which round gobies were most abundant.

[c] Annual average. Value of 2.19 in Vanni (2002) was an average of July–October.

gobies or a relatively low population biomass density. Several studies have directly measured mass-specific phosphate excretion by fishes and have found it to range from 0.5 to 21.4 μg P·g wet mass^{-1}·h^{-1} (Table 4). We derived mass-specific excretion rates from our simulated round gobies and found them to range from 0 to 2 μg P·g wet mass^{-1}·h^{-1} across all years. Zero excretion generally occurred in early May as growth resumed following weight losses during winter, and the highest excretion rates occurred during summer and autumn as water temperatures warmed and consumption increased. In general, round gobies in our model were excreting phosphorus at a lower rate than some direct measures but within the range of others (Table 4). Hence, the extremely low population excretion by round gobies is not fully explained by an extremely low mass-specific excretion rate.

A low round goby population biomass density, compared with previous studies, could be another explanation for the low population excretion. Among other species caught in the bottom trawls during our field sampling, round gobies were ranked either second or third in terms of mean biomass captured during August of 1997–2000 when their population was at its peak. As the population abundance declined in 2001 and 2002, however, their August biomass ranking declined to sixth and fourth, respectively. Among native species during 1997–2000, round goby biomass ranked below yellow perch (*Perca flavescens*) in each year and below freshwater drum (*Aplodinotus grunniens*) only in 1998. Among nonnative species, round goby biomass exceeded that of rainbow smelt (*Osmerus mordax*) during August 1998–2000. Thus, the trawl samples revealed round gobies to be an important component of the central Lake Erie benthic community, especially during 1997–2000. When considering the area of central Lake Erie, however, the highest annual mean biomass density was only 2.4 kg·ha^{-1} (230 individuals·ha^{-1}) in 1999. Most of the previous studies that have estimated fish excretion have focused on populations or assemblages with biomass densities >10 kg·ha^{-1}, and those with the highest excretion rates have biomass densities ranging from 199 to 417 kg·ha^{-1} (Table 4). Although round goby biomass was relatively high among species in the central basin, its biomass in comparison with other studies that have estimated fish excretion rates is extremely low. Hence, this relatively low round goby biomass density is likely responsible for the comparatively low population excretion rate.

Given the relatively low biomass density estimate, we consider the possibility that our sampling underestimated the round goby population size. First, we were not able to sample round gobies in depths <5 m. Because this depth strata represents <2% of the area of the central basin of Lake Erie (Haltuch and Berkman 1998), round gobies would have to attain extremely high densities for this omission to have a measurable impact on the total population density. Assuming that round goby density is influenced by habitat, we can assume that round goby density in 0–4.99 m depth is similar to that in 5–9.99 m depth owing to similar habitat composition (0–4.99 m: 40.7% glacial till, 36.2% sand–gravel, 17.2% bedrock, 5.9% sand–mud; 5–9.99 m: 51.1% glacial till, 24.5% sand–gravel, 15.8% bedrock, 8.6% sand–mud (Haltuch and Berkman 1998)). In 1999, when round gobies were most abundant, this assumption increased our total population estimate by only 2%, which is not nearly enough to push the biomass estimates up into the range of most other previous studies.

We also know that our population estimate was low because it did not include age-0 round gobies, which were not large enough to be collected in our bottom trawl. In terms of effects on phosphorus cycling, our model partially addressed the missing age-0 fishes, however. Age-1 round gobies in our model were simulated from 1 January to 31 December; field samples provided the abundance of age-1 round gobies on 1 June, and we used annual survival rates to back-calculate the abundance of that cohort to 1 January. Hence, we modeled round gobies that were only 6 months of age in all years, meaning that only the very youngest of gobies (i.e., 4–6 months of age) were not simulated in the model. Nonetheless, several studies have revealed the age-0 age-class to contribute a significant percentage of the overall population phosphorus recycling (e.g., 25%–50% (Kraft 1992, 1993; Chipps and Bennett 2000)). In our study, age-1 round gobies (which included fish as young as 4–6 months of age) also contributed more to phosphorus cycling than any other age-class. Undoubtedly, if our field sampling had provided estimates of age-0 round goby abundance and a bioenergetic model parameterized for larval round gobies were available, those model simulations would have revealed a higher population excretion rate for round gobies in the central basin of Lake Erie. Even if that rate were doubled (sensu Kraft 1993), however, the population excretion rate still would have been the lowest measured among other fish excretion studies.

Potential ecosystem effects in the Lake Erie central basin

Despite a low population excretion rate in comparison with other studies, we still wanted to evaluate the potential ecosystem-level effects of round gobies on the phosphorus cycling in the central basin of Lake Erie. One measure of the impact of phosphorus excretion by round gobies is to compare their excretion with the phosphorus required by primary producers. Before making this comparison, however, we needed to make some assumptions about whether the excreted phosphorus remained within the benthic habitat or diffused into the entire water column. The ultimate fate of these ions is dependent on a suite a factors, including whether or not the basin is stratified and the direction and magnitude of the prevailing winds and currents. Indeed, understanding the hydrodynamics is critical for the prediction of the ecosystem impact of dreissenid mussels on the phytoplankton community in the Great Lakes, as the mussels must rely on turbulent diffusion or "sinking" of phytoplankton to supply their food (e.g., MacIsaac et al. 1999; Ackerman et al. 2001). To maximize the potential impact of round goby excretion, we will assume that the phosphorus remains in the benthic habitat; if we had assumed that the phosphorus diffused into the entire water column, then we would have minimized the effects of round goby excretion because the phosphorus would diffuse into a much larger volume of water. At 2 m depth, the net benthic community photosynthetic rate in Lake Erie is ~30 mg C·m^{-2}·day^{-1} (R.E. Hecky, Biology Department, University of Waterloo, Waterloo, ON N2L 3G1, Canada, personal communication). Dividing by the Redfield ratio of carbon to phosphorus (106:1), we estimated that ~730 μg P·m^{-2}·day^{-1} is required to sustain benthic primary production. Because we could not sample 2 m depth areas, we will use our nearshore (between 5 and 15 m depth) round goby density estimate as a surrogate to calculate an areal excretion rate of round gobies at 2 m depth. In 1999, when the round goby abundance was at its peak, nearshore excretion was only 2.7 μg P·m^{-2}·day^{-1}, which is about 0.4% of the phosphorus required for benthic primary production. Thus, even in the scenario where round goby excretion was maximized by minimizing the volume of water in which the phosphorus diffused, round gobies were predicted to contribute little to primary production.

Another way to evaluate the impact of fish excretion is to compare it with the amount of phosphorus that enters the system from the watershed. In some reservoirs, phosphorus excretion by fishes can exceed the external phosphorus loading into the system during some parts of the year (Braband et al. 1990; Schaus et al. 1997; Gido 2002), although the biomass of those fish or fish assemblages ranged from 192 to 417 kg·ha^{-1}. The annual excretion by the round goby population peaked at 7.3 t in 1999, but that represented only 0.6% of the total phosphorus externally loaded to the central basin in that year (D. Dolan, Department of Mathematics, University of Wisconsin-Green Bay, Green Bay, WI 54311, USA, unpublished data). During 1997–2000, phosphorus recycled by round gobies was only 0.3%, on average, of the external phosphorus load to the basin. This analysis is another indication of the limited direct impact of round gobies on the Lake Erie phosphorus cycle.

We also evaluated the indirect effect of round gobies on phosphorus recycling through their consumption of dreissenid mussels. Since their invasion in the late 1980s, the filtering capacity of zebra mussels has been suggested to contribute to reductions of phytoplankton and increased water transparency in Lake Erie (e.g., MacIsaac et al. 1992; Madenjian 1995). With regard to nutrient regeneration, their high nutrient excretion rates also can have considerable lakewide impacts. Arnott and Vanni (1996) estimated phosphorus recycling by zebra mussels to be greater than all other sources, including zooplankton, sediment release, macrophytes, and external inputs. Because round gobies consumed up to 12 379 t of dreissenid tissue per year during our study period, we determined whether the "new" nutrients released from round goby consumption of dreissenid tissue was offset by a reduction in excretion of those mussels. Despite dreissenid mussels having a mass-specific excretion rate that is two orders of magnitude higher than round gobies (zebra mussels: 1.02 μmol P·g dry mass^{-1}·h^{-1} (Arnott and Vanni 1996); round gobies: 0.04 μmol P·g dry mass^{-1}·h^{-1} (this study)), excretion by round gobies that was attributed to consumption of dreissenids was 2.8 times higher, on average, than the potential excretion by those dreissenids. This result is a consequence of the wet mass of the round goby population being at least 100 times greater than the mass of the dreissenid mussels that were consumed on any given day. Nonetheless, taking the potential excretion into account diminishes the amount of new phosphorus that round gobies excrete from consuming dreissenid tissue.

Owing to the importance of dreissenid mussels in the phosphorus recycling in Lake Erie (Arnott and Vanni 1996), we also were interested in whether the round goby predators were making an impact on the population dynamics of dreissenid mussels. Unfortunately, we could not find a time series of dreissenid biomass in the central basin to determine whether increasing round goby densities coincided with reductions in dreissenids. In 1998, however, we were able to compare the consumption estimate of dreissenids by round gobies with an estimate of dreissenid biomass in the central basin (Jarvis et al. 2000). Even with a relatively high population density by round gobies in 1998, we found that they consumed about 0.1% of the dreissenid biomass in the basin. This result, coupled with sonar estimates that reveal increases in dreissenid percent cover within the central basin (Haltuch et al. 2000), suggests that round goby consumption is not having a basin-wide impact on the population dynamics of dreissenid mussels. As a result, round gobies do not appear to be indirectly affecting phosphorus cycling by reducing the biomass of dreissenids in the central basin.

Predictions regarding the impact of introduced species on phosphorus cycling

Our results revealed round goby to have a negligible impact on the phosphorus available to primary producers in the central basin of Lake Erie. This result is counter to several previous studies that have documented a considerable effect by introduced species on phosphorus cycling in lakes and reservoirs. In the 1970s, alewife excretion and egestion of phosphorus in Lake Michigan was estimated to be equivalent to phosphorus recycling by zooplankton (Kraft 1993). Excretion by tilapia in Lago Paranoá, Brazil, was estimated to be 9%–21% of the external phosphorus load (Starling et al. 2002), and excretion by trout in several Sierra Nevada lakes was estimated to be equivalent to the lake's atmospheric deposition of phosphorus (Schindler et al. 2001). Several factors may influence the impact of an introduced species on phosphorus cycling, including its population biomass density and the food web structure to which the fish are introduced.

In the case of tilapia in Lago Paranoá and alewife in Lake Michigan, both of these species attained high biomass densities (>300 and 69 kg·ha^{-1}, respectively) compared with round gobies in Lake Erie (≤ 2.4 kg·ha^{-1}), which likely explains the greater effect of nonnative alewife and tilapia in those systems. The food web within which the fish is introduced also may determine whether the fish influences nutrient cycling. Trout introduced into oligotrophic Sierra Nevada lakes had a considerable impact on phosphorus cycling (Schindler et al. 2001) despite reaching biomass densities that were relatively low compared with other fish excretion studies (Table 4). In most of these systems, the trout were introduced into systems that were historically without fish, so it is not surprising that they would have a measurable impact on phosphorus cycling. These salmonids recycled phosphorus through their consumption of zooplankters but also provided new sources of phosphorus by feeding on benthic and terrestrial prey that were not consumed by native organisms (Schindler et al. 2001). Conversely, the effect of zooplanktivorous kokanee salmon (*Oncorhynchus nerka*) on phosphorus cycling in Lake Pend Oreille was influenced by another species, *Mysis relicta* (opossum shrimp) (Chipps and Bennett 2000). In this food web, *Mysis* and kokanee both compete for zooplankton, but *Mysis* appear to have gained a competitive advantage, which contributes to their greater effects on phosphorus recyling (Chipps and Bennett 2000).

Overall, fish likely have the greatest impact on phosphorus cycling when introduced into systems with simple food webs (i.e., fishless Sierra Nevada lakes). If introduced into more complex food webs, their effects on phosphorus cycling will depend on the outcome of competitive or predatory interactions (i.e., Lake Pend Orielle). If the fish are successful enough to reach extraordinarily high biomass densities (i.e., alewife in Lake Michigan or tilapia in Lago Paranoá), then that biomass alone will have some impact on the phosphorus cycles in lakes. Despite their ability to take advantage of abundant dreissenid mussels as a prey resource, round gobies in the central basin of Lake Erie did not attain sufficiently high biomass densities to influence phosphorus cycling. It remains to be determined whether their population has been limited by piscivores in the lake, as round gobies have become prevalent in the diets of smallmouth bass (*Micropterus dolomieu*), yellow perch, white bass (*Morone chrysops*), walleye (*Stizostedion vitreum*), and burbot (*Lota lota*) in Lake Erie (C.T. Knight, unpublished data).

In conclusion, Lake Erie remains a dynamic ecosystem whose nutrient dynamics are difficult to understand owing to its interannual variability in external nutrient inputs and the seemingly constant stream of invaders. With the success of introduced dreissenid mussels, whose filtering has contributed to water clarity and facilitated the resurgence of benthic algae and invertebrates, Lake Erie is believed to have shifted from a pelagic- to a benthic-dominated system (Johannsson et al. 2000). From an ecosystem perspective, dreissenid mussels can increase dissolved nutrient concentrations in the water column through excretion (Heath et al. 1995). As a benthic fish, the round goby has been contributing to the increasing biomass of the benthic habitat, but heretofore, its ecosystem impacts were unknown. Our model results indicated that round goby effects on phosphorus cycling in the central basin of Lake Erie are minimal. Directly, their excretion contributes little (0.4%) to benthic primary production. Their population excretion estimates were lower than those of any previously published studies, a result of their comparatively low population biomass density. Indirectly, round gobies consumed far less than 1% of the dreissenids in central Lake Erie, indicating that round gobies have not been influencing phosphorus cycling by reducing the abundance of the dreissenid population, the most important nutrient recycler in the lake (sensu Arnott and Vanni 1996). In our view, future research regarding round goby ecosystem effects should focus on their role as couplers of the benthic and pelagic food web. If round gobies remain prevalent in the diets of pelagic piscivores, then they will serve as a vector to move energy and nutrients concentrated in the benthic habitat up into the pelagic habitat.

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Late-season mortality during migration of radio-tagged adult sockeye salmon (*Oncorhynchus nerka*) in the Columbia River

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Abstract: We radio-tagged 577 adult sockeye salmon (*Oncorhynchus nerka*) returning to the Columbia River in 1997 to determine how migration behaviors were related to migration success in an altered river system. The probability of successful migration declined dramatically for late-entry individuals, concomitant with declines in discharge and the onset of stressful temperatures. Long dam passage times were not related to unsuccessful migration at most dams. However, when migration histories were analyzed across multiple dams or reservoirs, relatively slow migration was significantly associated with unsuccessful migration, suggesting potential cumulative effects. Median passage times at dams were rapid (7.9–33.4 h), although 0.2%–8% of salmon took more than 5 days to pass. Reservoir passage was also rapid, averaging 36.8–61.3 km·day^{-1}, and appeared to compensate for slowed migration at dams. Rates observed in the unimpounded Hanford Reach suggest that total predam migration rates may have been similar to current rates. Overall, our results suggest that cumulative effects may be more important than negative effects of passage at single dams and that hydrosystem alteration of temperature regimes in the migration corridor may have an important indirect negative impact on adults.

Résumé : Nous avons muni d'émetteurs radio 577 saumons rouges (*Oncorhynchus nerka*) qui retournaient dans le Columbia, afin de déterminer de quelle manière les comportements migrateurs sont reliés au succès de la migration dans un système hydrographique modifié. La probabilité d'une migration réussie diminue de façon spectaculaire chez les individus qui arrivent tard, au moment où le débit diminue et où les températures accablantes s'installent. Dans la plupart des barrages, la durée prolongée de traversée n'est pas reliée à l'insuccès de la migration. Cependant, lorsque le parcours de la migration au travers plusieurs barrages et réservoirs est analysé, il y a une relation significative entre une migration relativement lente et l'insuccès de la migration, ce qui indique la possibilité d'effets cumulatifs. La durée médiane du passage des barrages est courte (7,9–33,4 h), bien que 0,2–8 % des poissons mettent plus de 5 jours à les traverser. La traversée des réservoirs est aussi rapide, à une vitesse moyenne de 36,8–61,3 km·jour^{-1}, et semble compenser le ralentissement de la migration aux barrages. Les taux observés dans la section Hanford qui ne contient pas de réservoir indiquent que les taux de migration avant l'érection des barrages ont dû être semblables aux taux actuels. En gros, nos résultats indiquent que les effets cumulatifs peuvent être plus importants que les effets négatifs causés par le passage des barrages individuels et que la modification des régimes thermiques du système hydrographique dans le corridor de migration peut avoir un important impact négatif indirect sur les adultes.

[Traduit par la Rédaction]

Introduction

Returns of wild Pacific salmon (*Oncorhynchus* spp.) and steelhead (*Oncorhynchus mykiss*) in the Columbia River basin have declined dramatically from historic levels (Raymond 1988; McClure et al. 2003). Declines have been attributed primarily to development of the Columbia River for hydroelectric power, overharvest, habitat loss, and hatcheries (Raymond 1988; National Research Council 1996; Ruckelshaus et al. 2002). Currently, 12 Columbia basin salmon and steelhead evolutionarily significant units are listed as endangered or threatened under the US Endangered Species Act (National Marine Fisheries Service 2000), including one sockeye salmon (*Oncorhynchus nerka*) run. Hydroelectric dams have transformed the river into a series of reservoirs punctuated by dams extending through much of the migra-

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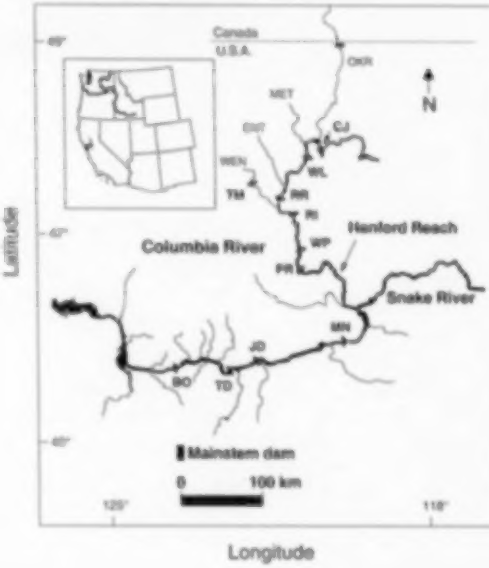
tion corridor. Despite this dramatic change, relatively little is known about the behavior and survival of adult salmon, particularly sockeye salmon, as they migrate upstream, and there is concern that dams may slow migration, subsequently reducing escapement to spawning grounds, population viability, and species recovery (Quinn et al. 1997; Dauble and Mueller 2000).

The construction of Grande Coulee Dam in 1939 blocked sockeye access to habitat in the upper Columbia River, and consequently, the majority of Columbia basin sockeye salmon stocks went extinct (Nehlsen et al. 1991). Adult sockeye salmon enter the Columbia River in early summer (Quinn et al. 1997) with peak counts at Bonneville Dam primarily in June and July and spawn in tributaries in September and October. Columbia River sockeye salmon stocks are primarily wild, native and are found in few drainages in the basin (Mullan 1986; Fryer 1995). Currently, most sockeye salmon spawn in tributaries to Lakes Wenatchee (Wenatchee River basin) and Osoyoos (Okanogan River basin) (Fig. 1) in September and October (Mullan 1986; Quinn et al. 1997). These lakes are 842 and 946 km from the mouth of the Columbia River, respectively, and comprise <4% of the historical sockeye salmon nursery habitat in the upper Columbia River basin (Gustafson et al. 1997). These stocks are not listed under the Endangered Species Act, although Nehlsen et al. (1991) classified the stocks as of special concern. The Snake River sockeye salmon evolutionarily significant unit is listed as endangered because of critically low population size, and thus, the Wenatchee River and Okanogan River evolutionarily significant units account for nearly all remaining sockeye salmon in the Columbia basin (Quinn and Adams 1996). The Wenatchee and Okanogan stocks pass seven and nine dams, respectively, to reach spawning tributaries.

In addition to representing a migration barrier, there is concern that dams and other forces including changes in land use and regional climate have altered the thermal regime encountered by migrating salmon. Temperatures >20 °C are physiologically stressful to salmonids (Becker and Fujihara 1978; McCullough et al. 2001), including sockeye salmon (Brett 1965; Gilhousen 1990), and temperatures above 24 °C are thought to be lethal to migrating adult salmon (Servizi and Jensen 1977). Warm summer temperatures are thought to constrain the run timing of salmon (e.g., Quinn and Adams 1996; Hodgson and Quinn 2002), and water temperatures in the mainstem Columbia River consistently exceed these values in summer. In particular, indirect data from historical dam counts support the hypothesis that earlier warming and higher summer temperatures over the last half century have led to earlier run timing in Columbia River sockeye salmon (Quinn and Adams 1996; Quinn et al. 1997). Many factors may contribute to the warming trend of the Columbia River including irrigation and land use practices (Coutant 1999), heating caused by greater residence time of water in impoundments (Quinn and Adams 1996), and perhaps regional climate change (Neitzel et al. 1991; Hamlet and Lettenmaier 1999).

We used our observational telemetry data to address several questions related to basic salmon biology and the management of salmon in an impounded system. We asked the following. Was migration success (defined as survival to a spawning tributary) related to behavior during dam passage and (or) environmental conditions encountered during migration? What was the seasonal pattern of migration success? What were the general daily and seasonal patterns of migration and how were environmental factors related to dam passage and reservoir migration behavior? How did migration behavior at dams and in reservoirs compare? We also discuss how migration success and straying compared with sockeye migration in other river systems and with migration by other Columbia River salmon species. Finally, we use migration data from a single 70-km unimpounded reach to speculate about whether current migration rates through the hydrosystem differed from the historic condition.

Fig. 1. Map of the study region in the northwestern United States (inset) including the location of radio receivers at dams and major tributaries within the Columbia River study area in 1997. Receiver locations at mid-Columbia River and tributary sites are maintained by the Chelan and Douglas County public utility districts. Dam abbreviations: BO, Bonneville; TD, The Dalles; JD, John Day; MN, McNary; PR, Priest Rapids; WP, Wanapum; RI, Rock Island; RR, Rocky Reach; WL, Wells; TM, Tumwater; CJ, Chief Joseph. Tributary abbreviations: WEN, Wenatchee River; ENT, Entiat River; MET, Methow River; OKR, Okanagan River.

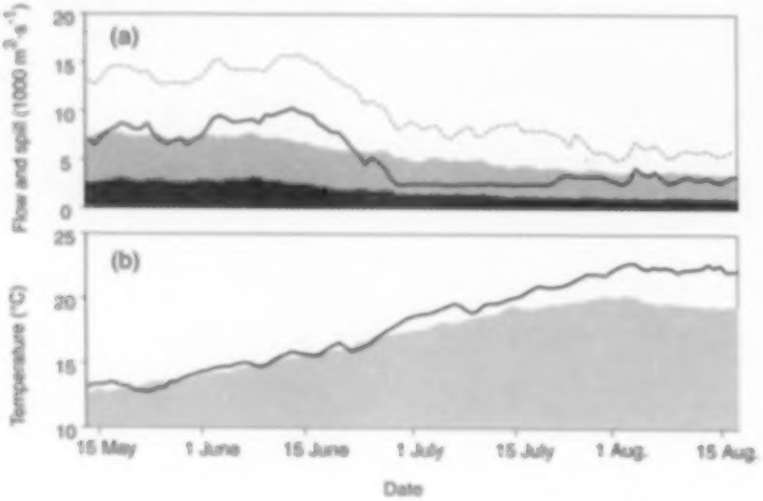


Methods

Study system

The Columbia River study area included four mainstem dams and reservoirs in the lower Columbia River (Bonneville, The Dalles, John Day, and McNary) and five mid-Columbia River dams and reservoirs (Priest Rapids, Wanapum, Rock Island, Rocky Reach, and Wells) and all major tributaries between Bonneville and Chief Joseph dams on the Columbia River (Fig. 1). The University of Idaho Cooperative Fish and Wildlife Research Unit maintained radio receivers and antennas at dams and tributaries between Bonneville and Wanapum dams, while concurrent telemetry projects by the

Fig. 2. Plots of (*a*) mean river discharge and discharge over the spillway and (*b*) temperature conditions at Bonneville Dam from 1987 to 1996 and during 1997. Fig. 2*a*: the broken line represents flow, the solid line represents spill, shading represents 1987–1996 flow, and bars represent 1987–1996 spill. Fig. 2*b*: the line represents 1997 temperature and shading represents 1987–1996 temperature. The seasonal patterns of flow, spill, and temperature conditions at the other Columbia River dams were similar. Both discharge and spill during the study period were unusually high at all dams.



Chelan and Douglas County public utility districts (Alexander et al. 1998) provided data from Rock Island, Rocky Reach, Wells, and Chief Joseph dams. The University of Idaho Cooperative Fish and Wildlife Research Unit also monitored mainstem and tributary sites with mobile radio-tracking units, and additional data were collected from hatcheries, fisheries and spawning ground surveys. All Columbia River stocks of sockeye salmon tagged at Bonneville Dam could therefore be tracked to spawning tributaries.

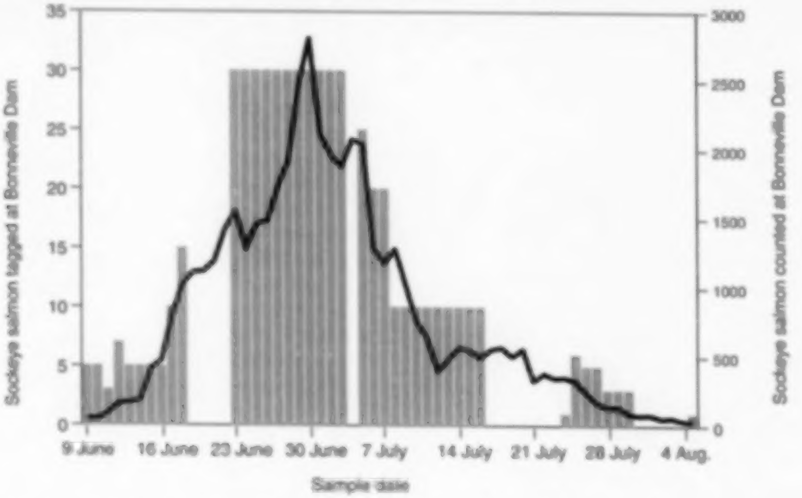
River conditions during the 1997 study were characterized by high flows. Mean daily Columbia River discharge in June and July was 10 724 m^3·s^{-1}, nearly double the 10-year average (1987–1996) of 5635 m^3·s^{-1}. Consequently, discharge over the spillway of dams (spill) during the sockeye salmon migration was often several times higher than average (Fig. 2). Water temperatures in the lower Columbia River ranged from about 13 °C in early June to 23 °C in late August, levels that were near average (Fig. 2). Mean summer water temperatures in the mid-Columbia River upstream from the Snake River confluence averaged 3–5 °C cooler than in the lower Columbia River. Despite the high discharge conditions, water temperatures were warmer than average after June (Fig. 2).

Trapping and tagging

Adult sockeye salmon were trapped for intragastric radio-tagging (Mellas and Haynes 1985) in the adult fish facility adjacent to the Washington-shore ladder at Bonneville Dam as they migrated upstream using the techniques described in greater detail by Keefer et al. (2004*a*). The fish were radio-tagged using 3-V radio transmitters (Lotek Wireless, Inc., Newmarket, Ontario) that transmitted a unique digitally coded signal every 5 s. Radio-tags were cylindrical and weighed 11 g in air (4.3 cm × 1.4 cm). After tagging, fish were moved to a 2275-L oxygenated, insulated transport tank and were then released within 6.2 h (mean = 1.96 h, SD = 1.05 h, range = 0.43–6.20 h) at locations 9.5 km downstream from Bonneville Dam on both sides of the river. No sockeye salmon mortalities occurred during tagging, transport, or release.

We radio-tagged 577 sockeye salmon between 9 June and 5 August approximately in proportion to daily run counts (Fig. 3). The tagged fish represented 1.2% of the 46 665 sockeye salmon counted passing Bonneville Dam during the tagging period. We tagged all fish regardless of minor injury or fin clip. Fish with severe injuries (e.g., gashes penetrating to the coelomic cavity or those that affected swimming performance) accounted for <1% of all fish trapped and were not tagged. The exclusion of these fish may have led to a slight (<1%) overestimation of survival, but we do not expect that this bias would affect our estimates of migration behavior. While we selected fish haphazardly and tagged them in approximate proportion to the number passing the dam each day, the samples were not truly random because only fish passing through one of the two Bonneville ladders were sampled. Further, the proportion sampled each day varied slightly, and we did not sample at night because few sockeye salmon pass dams at night (Quinn and Adams 1996; Results). We estimated that 72% of the 577 radio-tagged fish were male and 28% were female, a sex ratio typical in returning sockeye (e.g., Gustafson et al. 1997). Sixty-nine percent of the fish that entered tributaries were males (n = 267) compared with 31% female (n = 121), proportions similar to those at the time of tagging (χ^2 test, p = 0.24, n = 574). Five fish (0.9%) had adipose or ventral fin clips, and 99.1% were unclipped, presumably wild fish. Fork lengths of tagged fish ranged from 38 to 63 cm (median = 49.5 cm) and mean fork lengths of radio-tagged sockeye salmon that entered

Fig. 3. Number of sockeye salmon outfitted with radio transmitters at the Bonneville Dam adult fish facility (bars) and the number counted passing the dam at the counting stations (line) during the migration in 1997.



Wenatchee tributaries (including White River fish, mean = 50.3 cm, range 38–61 cm) were slightly larger than those entering the Okanogan River (mean = 48.8 cm, range 43–56 cm; separate variances *t* test, $t = -5.074$, $p < 0.001$, $n = 384$).

Telemetry monitoring and fate assignment

Fixed radiotelemetry sites were the primary means of assessing movements and passage rates of radio-tagged sockeye salmon. We used SRX receivers (Lotek Wireless, Inc.) with aerial Yagi antennas to monitor tailrace areas at dams and tributary mouths and digital spectrum processors added to SRX receivers with underwater antennas made of coaxial cable to monitor fishways and ladder exits at dams. On average, receivers operated >90% of the time at lower Columbia River dams and >80% of the time at Priest Rapids and Wanapum dams (Table 1). Trucks and boats outfitted with aerial antennas were used to track fish in areas not covered by fixed-site receivers, including mainstem reservoirs, accessible tributaries, and the Hanford Reach below Priest Rapids Dam. At the Wenatchee River, fixed site receivers were deployed at the mouth and at Tumwater Dam (53 km upstream from the mouth). Mobile tracking upstream from Wells Dam was conducted approximately every 2 weeks from late July to early November, a period much shorter than the typical period between arrival in the Okanogan River and the initiation of spawning (Hodgson and Quinn 2002).

We used a combination of telemetry records, spawning ground surveys, and radio-tag returns from fisheries to classify fish as successful migrants or as mortalities. Successful migrants included salmon with telemetry records in known spawning tributaries, those found as carcasses in spawning tributary surveys, or those that returned to their hatchery of origin. Mortalities included returns from fisheries and salmon whose final telemetry records were outside spawning tributaries, usually between dams. We felt that these operational definitions represented the true fates of fish for several reasons. First, the spatial distribution of spawning habitat of sockeye in the Columbia basin is highly restricted relative to other salmon species (Mullan 1986; Groot and Margolis 1991; McClure et al. 2003). Hence, successful spawning in unmonitored tributaries or the mainstem Columbia River was unlikely. Second, the rate of transmitter loss through regurgitation was low (<1%, $n = 3$), a similar rate observed in other studies (Ramstad and Woody 2003; Keefer et al. 2004*a*) and these fish were not considered in further analyses. Third, the linear nature of the study system and large number of receivers used (Table 1) provided a high probability of detection, particularly at dams. For instance, we estimate the probability of a fish last recorded in the Hanford Reach reaching a spawning tributary by passing the next two dams undetected at $<1.0 \times 10^{-7}$ based on the minimum number of fixed site receivers that salmon passed at these dams ($n = 8$) and frequencies of receiver outage at these sites (mean outage = 14.0%, SD = 6.0%). Thus, while many fish went undetected at individual antennas, it was improbable that misclassification of fates seriously biased our results overall.

Reach survival estimates

In addition to overall survival to spawning tributaries, we estimated survival through each hydrosystem segment. Reach-specific survival (S_i) was calculated with the formula

$$S_i = (N_{i+1} + T_i)/N_i$$

where N_{i+1} is the number of fish that passed the upstream dam, T_i is the number of fish that entered spawning tributaries in the reach above dam *i*, and N_i is the number of fish that passed the downstream dam. Fish that passed the dam at the upstream end of a reach were considered to have survived through the reach regardless of subsequent downstream movement. Fish last recorded in tributaries downstream from known spawning habitat (all tributaries downstream from

Table 1. Location of receivers at dams and tributaries with number of antennas and percent time in operation in 1997.

Receiver site	Receiver location	No. of antennas	% time in operation
Bonneville Dam	Tailrace	2	95.5
	Dam	68	92.5
	Ladder	33	96.2
	Navigation lock	5	96.6
The Dalles Dam	Tailrace	2	89.9
	Dam	6	97.9
	Ladder	15	96.9
John Day Dam	Tailrace	2	98.7
	Dam	3	99.9
	Ladder	17	94.8
McNary Dam	Tailrace	2	91.7
	Dam	38	94.5
	Ladder	17	96.8
	Navigation lock	6	98.8
Priest Rapids Dam	Tailrace	2	84.8
	Dam	22	93.4
	Ladder	13	85.2
Wanapum Dam	Tailrace	2	82.8
	Dam	18	96.3
	Ladder	12	95.3
Bridge of the Gods	Bonneville Dam forebay	1	96.2
Wind River	Mouth	1	93.6
Little White Salmon River	Mouth	1	94.1
White Salmon River	Mouth	1	88.9
Hood River	Mouth	1	98.3
Klickitat River	Mouth	1	97.3
Deschutes River	Mouth	1	98.7
John Day River	Mouth	1	85.3
Umatilla River	Mouth	1	99.9
Walla Walla River	Mouth	1	97.8
Yakima River	Mouth	1	95.7
Hanford Reach	Right and left banks	2	88.3

Rock Island Dam) were considered strays that did not survive to spawning areas.

Passage time calculations

We used tailrace and top-of-ladder sites for calculating dam and reservoir passage times. Dam passage times were calculated from the first record at a tailrace receiver (0.5–3.2 km downstream from dams) to the last record at a top-of-ladder receiver. Some fish migrated back out of tailraces, a behavior that we believe was related to route searching or unfavorable passage conditions at dams, and dam passage times included this time. Reservoir passage times were calculated from the last top-of-ladder record at the downstream dam to the first tailrace record at the upstream dam of each reservoir. Reservoir passage times were converted to daily rates using river kilometres because reservoir lengths varied. Time that sockeye salmon temporarily strayed into tributaries was included in total reservoir passage time, but did not include time fish spent downstream from dams after fallback events (downstream movement through turbines, juvenile bypass facilities, fish ladders, or navigation locks or over spillways; Reischel and Bjornn 2003). Sample sizes for dam passage and reservoir migration times varied between sites because of mortalities between dams and loss of receiver coverage resulting primarily from power outages or vandalism. In particular, we could not calculate passage times for 254 fish at McNary Dam (64% of all passages) or 259 fish at Priest Rapids Dam (60% of all passages), because they were recorded near top-of-ladder sites but missed the uppermost antennas because of receiver outages.

Environmental data and statistical analysis

We performed statistical analyses to assess the relationships between migration performance and behavior and environmental variables at several scales. First, we used binary logistic regression (Hosmer and Lemeshow 1999) to test whether environmental conditions, relative migration speed, or fallback history was associated with migration success. Prior to the regression analysis, we used principal components analysis (PCA) to summarize variation in five predictors because of strong intercorrelation among environmental predictors. The PCA included mean daily temperature and discharge at Bonneville Dam on the day of tagging and tagging date as a potential cue signaling the end of the spawning season (pairwise Pearson's $r^2 = 0.620–0.897$, $p \le 0.001$) (e.g., Fig. 2). Mean relative passage times and migration rates were used to estimate the relative migration speed in the lower Columbia River among sockeye salmon and were estimated by taking the mean of the normal deviates (Zar 1999) for passage time or migration rate through each lower Columbia River project. The PCA scores for each fish were then used as predictors in the logistic regression model. The fallback history of each fish was included as an additional categorical predictor by classifying fish as having fallen back over one or more dams or not. A Hosmer–Lemeshow test tested the goodness-of-fit of the logistic regression model to the data using the "smart = 10" option in Systat v.10 (Steinberg and Colla 1997). We used the mean daily flow and temperature at the time the fish first entered each dam tailrace obtained from the US Army Corps of Engineers (compiled by the University of Washington at http://www.cqs.washington.edu/dart/dart.html). No interaction terms were significant in preliminary models ($p > 0.10$) and were subsequently excluded.

At a finer scale, we examined migration behavior at dams and through reservoirs in relation to daily and seasonal variation in environmental conditions. We used event-time analysis (also known as survival or failure-time analysis; Hosmer and Lemeshow 1999; Castro-Santos and Haro 2003) to examine the relationship between passage time at each dam and predictor variables describing environmental conditions and fish traits. A Cox proportional hazards regression model in the form

$$h_{i(t)} = \lambda_0(t) \exp[\beta_1 x_{i1} + \ldots + \beta_k x_{ik}(t)]$$

where $h_{i(t)}$ is the probability or "hazard" of passage of individual i at time t, given that an individual had not passed prior to time t. The model relates individual hazards to a baseline hazard function $\lambda_0(t)$, which is left unspecified. The model then fits a set of regression coefficients β_{ij} by maximum likelihood using a set of predictor covariates $x_{i1},\ldots, x_{ij}$ describing environmental conditions and fish traits for each fish, and these covariates may be constant (e.g., fish length at tagging) or time varying (e.g., light level at the time of passage). The Cox proportional hazards models have several advantages over traditional analysis of variance (ANOVA) approaches or other event-time models (Allison 1995; Castro-Santos and Haro 2003). First, individuals that are recorded at the tailrace of a dam but that do not pass can be explicitly included in the modeling. These individuals are modeled as "at risk of passage" at the time of tailrace detection and remain in the risk set until censoring, as described below. Second, the covariates may vary through time, allowing passage hazards to change daily or seasonally. The primary disadvantage is that the models are semiparametric in the sense that hazards are calculated using the ranks of covariate values, and consequently, quantitative differences among treatments cannot be modeled as in traditional regression (Allison 1995; Castro-Santos and Haro 2003). Hazard ratios compare the probability of the event occurring within a given time interval for an individual belonging to one group versus another (e.g., females versus males) or for an increase in one unit of a continuous predictor variable. The models included the length of each fish at the time of tagging as a measure of body size, fish sex, and two time-varying covariates: the date of passage and time of day, a binary variable coding for day versus night. The date of passage for each fish at each project was used as a compound measure of seasonal changes in temperature and discharge and as a predictor of seasonal effects on migration behavior owing to the high intercorrelations among these variables as indicated by unstable parameter estimates and extremely large standard errors in models incorporating multiple environmental predictors (unpublished analyses; Allison 1995). Models replacing passage date with mean daily discharge, spill, or temperature produced qualitatively identical results. The daily time of switching between day and night was estimated using sunrise–sunset times at Stevenson, Washington, obtained from the US Naval Observatory (http://aa.usno.navy.mil/data/docs/RS_OneYear.html), because passage is known to slow substantially at night (Quinn and Adams 1996) (see Results) and to account for changes in day length during the run. All proportional hazards regressions were performed using SAS v.8e (SAS Institute Inc., Cary, N.C.) with the efron option for breaking ties (Allison 1995). Fish were censored if they (*i*) were detected at the tailrace antenna but did not pass the dam, (*ii*) passed the dam (i.e., they were detected upstream) but were not detected at the top-of-ladder antenna, or (*iii*) passed via the navigation lock. We used data from other dam antennas to assign censoring times (the time when fish were lost to followup) or, when unavailable, the censoring time equaled the maximum observed passage time. Reported p values are uncorrected for multiple comparisons across dams.

For the event-time models, we focused on the relationship between passage time (as modeled by hazard of passage) and predictor variables at individual dams because of the potential importance of passage facilities at each hydroelectric project in the management of this species. We also used a repeated-measures, mixed-model ANOVA followed by a series of contrasts to examine whether mean passage times differed among hydroprojects. The repeated-measures model related the mean passage time observed at each dam to migration success, the date of tagging, and the time of day that fish entered the tailrace of the dam because there was a strong diel periodicity observed in passage behavior (see Results). We then used contrasts to test (*i*) whether mean passage times differed at lower versus mid-Columbia River projects and (*ii*) whether mean passage time differed among dams within region. All dam passage time analyses were restricted to first passage attempts (i.e., excluding fallback time) and excluded known hatchery (clipped) fish. Passage times were $\log_e$-transformed to improve normality and homogeneity of the error terms.

We assessed the rate of migration through reservoirs and the Hanford Reach using a similar repeated-measures ANOVA model and contrasts. An additional contrast tested whether migration rates through the Hanford Reach differed from those through all reservoirs. The appropriateness of four different covariance structure types (unstructured, compound symmetric, autoregressive, and heterogeneous autoregressive) was compared for both the passage time and the migration rate ANOVA models using the Akaike information criterion (Burnham and Anderson 2002); these comparisons suggested improvements in model fit using a heterogeneous autoregressive structure in both cases.

We compared the proportion of time successful migrants spent passing dams, ascending reservoirs, passing the Hanford reach, or in other activities (e.g., reascending dams after fallback) for 12 fish that had complete records at all tailrace and top-of-ladder antennas. A Freidman test was used to test for differences in mean proportions (Zar 1999). Qualitatively identical results were obtained using records of fish (*n* = 90) that passed Rock Island Dam, had complete passage time records at four or more dams, but were not detected at one of the 20 tailrace or top-of-ladder receivers required to record a complete record. The fallback rate for the sockeye salmon among complete-record fish (25.0%, *n* = 12) was nearly identical to that for all fish (25.6%, *n* = 574).

Results

Patterns of survival, straying, and migratory timing

Approximately two thirds of the radio-tagged sockeye salmon reached spawning tributaries (Table 2). About 41% of the fish that passed Rock Island Dam entered tributaries of Lake Wenatchee. Most of the fish passing Wells Dam (*n* = 231) were last recorded in the Okanogan River. Approximately 11% (*n* = 65) of the radio-tagged sockeye salmon were reported as harvested in mainstem Columbia River fisheries. Sockeye salmon with last records at a mainstem dam or reservoir comprised 18% (*n* = 100) of all radio-tagged fish; these fish presumably represent nonfisheries mortality. Reach survival estimates for sockeye salmon in 1997 exceeded 90% through all sampled reaches in the Columbia River hydrosystem except for the reach between Bonneville and The Dalles dams (87.4%) (Table 2).

Sockeye salmon took approximately 3 weeks to pass through the Columbia River hydrosystem. The migratory timing of the Wenatchee and Okanogan River stocks did not differ (Kolmogorov–Smirnov two-sample test, $p = 0.52$). Sockeye salmon that returned to these drainages were radio-tagged at Bonneville Dam primarily from mid-June to mid-July with median tag dates of 29 June for both stocks. Although the sample size was small (*n* = 10), fish bound for the White River drainage (Wenatchee River tributary) passed Bonneville Dam from late June through mid-July (median tag date 3 July). Median passage date at Rock Island Dam was 18 July (range 2 July – 16 August) with a median time of 20 days after tagging. Median arrival dates upstream at Wells Dam were 2 days later for Methow and Okanogan River stocks (20 July, range 5 July – 18 August).

Migration success was related to both seasonal environmental variation and relative migration speed. The PCA of variation in environmental variables and two measures of relative migration speed produced two axes corresponding primarily to variation in date, discharge, and temperature (axis 1, season) and relative migration rate in the lower Columbia (axis 2, speed) (Table 3). The fate of each fish was significantly related to both of these summary predictor variables. Adults that entered late and that encountered lower flows and higher temperatures were less successful. Relatively slow migration rates (slow migration through reservoirs and long passage times at dams in the lower Columbia; Table 3) were also associated with unsuccessful migration. The strong intercorrelations between tagging date and environmental factors made it impossible to relate variation in migration success to environmental factors versus phenological changes in behavior among individuals within the run. Migration success was marginally significantly related to fallback history (Table 4). Contrary to expectation, adults that fell back were more likely to successfully migrate. The Hosmer–Lemeshow test suggested that the model was an adequate description of the data ($\text{H–L}_{(8)} = 6.062$, $p = 0.640$) and correctly classified the fate of 70.4% of the fish. Most notably, only a single salmon of 27 (3.7%) tagged at Bonneville Dam during the period 24 July – 5 August successfully reached a spawning tributary (Fig. 4), concomitant with the onset of stressful river temperatures. In comparison, 61.3% of salmon tagged during the period 5–16 July were successful migrants (*n* = 155).

Passage of dams, reservoirs, and the Hanford Reach

We used ANOVA and proportional hazards regression to test for relationships between passage time and fish traits, environmental conditions, and fate at two scales: throughout migration and at individual dams. Passage times differed among most dams within region and were longer at mid-Columbia than at lower Columbia River dams (Fig. 5; Table 5). Distributions of passage times at all dams were skewed because some fish had long passage times, with 2%–8% taking 5 days or more to pass (Fig. 5). Mean passage times at dams decreased significantly during the migration season when assessed across dams (Fig. 6; Table 6), concomitant with increasing temperatures.

When statistically controlling for variation among individuals, dams, season, and arrival date at the tailrace (Table 5), the mean passage time of unsuccessful migrants was 13.7% slower than that of successful migrants based on back-transformed means (mean successful = 17.8 h, mean unsuccessful = 20.3 h). In contrast, the effect of fate was not significant at any of the dams except Bonneville Dam in the partial hazards regressions (Table 6), suggesting a cumulative effect of slow passage on migration success.

There was a strong diel periodicity in passage times (Fig. 7), and the time of arrival at the tailrace affected passage time because only about 10% of sockeye salmon passed top-of-ladder receivers at dams at night (2100–0500) (Fig. 8). Consequently, within a given time interval, the probability of passage at night was 0.093–0.308 that of passage during the day, i.e., passage during the day was 3.24–10.7 times more likely than at night (Fig. 7; Table 6). Additionally, passage hazard ratios declined with fish size, corresponding to faster passage times in larger fish at one lower Columbia and three out of four mid-Columbia projects. Sex was not related to passage hazard at any location (Table 6).

Migration rates in reservoirs were also related to seasonal changes in conditions as measured by tagging date and to migration success. Rates were rapid, ranging from mean rates of 37.8 km·day^{-1} at the Rock Island Reservoir to 61.3 km·day^{-1} at the John Day Reservoir (Fig. 9), and sockeye salmon migrated more rapidly through lower Columbia River reservoirs than through mid-Columbia River reservoirs, with mean rates 10.7–24.4 km·day^{-1} faster through lower Columbia reservoirs (Table 7; Fig. 9). Most pairwise comparisons within region were significantly different (Table 7). Overall mean reservoir migration rate was 3.3 km·day^{-1} (7.25%) higher in successful migrants than in unsuccessful migrants, primarily because of a decrease in migration rate in late-season, unsuccessful migrants (Table 7;

Table 2. Fate and reach survival estimates for sockeye salmon released downstream from Bonneville Dam in 1997.

Reach			Survived in reach (%)			Did not survive in reach (%)				Cumulative (%)	
Downstream	Upstream	Entered reach	Passed upstream dam	Spawning tributary	Total	Mainstem harvest	Stray	Unknown	Total	Survival	Mortality
Bonneville	The Dalles	563	87.4 (492)		87.4	9.2 (52)	0.9 (5)	2.5 (14)	12.6	87.4	12.6
The Dalles	John Day	492	95.1 (468)		95.1	1.0 (5)[a]	0.6 (3)[b]	3.3 (16)	4.9	83.2	16.8
John Day	McNary	468	97.6 (457)		97.6		0.2 (1)[c]	2.1 (10)	2.4	81.2	18.8
McNary	Priest Rapids	457	94.7 (433)		94.7	1.8 (8)	0.2 (1)	3.3 (15)	5.3	76.9	23.1
Priest Rapids	Wanapum	433	98.6 (427)		98.6			1.4 (6)	1.4	75.8	24.2
Wanapum	Rock Island	427	97.9 (418)		97.9			2.1 (9)	2.1	74.2	25.8
Rock Island	Rocky Reach	418	57.4 (240)	41.4 (173)	98.8			1.7 (5)	1.2	73.4	26.6
Wenatchee River				(163)							
White River				(9)							
Napeequa River				(1)							
Rocky Reach	Wells	240	96.3 (231)	1.7 (4)	97.9			2.1(5)	2.1	72.5	27.5
Entiat River				(1)							
Wenatchee River[d]				(3)[d]							
Wells	Chief Joseph	231	na	91.3 (211)	91.3			8.7(20)	8.7	68.9	31.1
Methow River				(3)							
Okanongan River				(208)							
Fate totals				68.9 (388)		11.5 (65)	1.8 (10)	17.8 (100)		68.9	31.1

Note: "Entered reach" indicates that fish passed the downstream dam. Sample sizes are given in parentheses. na, not available.

[a] One fish fell back over The Dalles Dam and was captured in a tribal fishery downstream from The Dalles Dam.

[b] Fell back over The Dalles Dam and entered tributaries downstream from The Dalles Dam.

[c] Fish fell back over The Dalles and John Day dams and entered a tributary downstream from The Dalles Dam.

[d] Three fish fell back at Rocky Reach Dam and entered the Wenatchee River downstream from Rocky Reach Dam.

Table 3. Component loadings from PCA of variation in tagging date, temperature, and discharge at Bonneville Dam on the date of tagging and relative passage time and migration rate through the lower Columbia River hydrosystem.

Factor	PCA1 (season)	PCA2 (speed)
Tag date	0.962	0.214
Temperature	-0.937	-0.023
Discharge	0.919	0.284
Relative passage time	-0.319	0.723
Relative migration rate	0.410	-0.628
% of variance explained	54.1	25.2

Table 4. Results of multiple logistic regression test for relationships between probability of successful migration and seasonal changes in environmental conditions, relative migration speed, and fallback history.

Parameter	Estimate	SE	*t* ratio	*p*
Constant	1.561	0.149	10.500	<0.001
PCA1 (season)	-0.721	0.120	-6.035	<0.001
PCA2 (speed)	0.468	0.118	3.969	<0.001
Fallback history	0.497	0.255	-1.945	0.052

Fig. 4. Histogram representing the number of sockeye salmon tagged by date for successful (solid bars) and unsuccessful migrants (open bars).

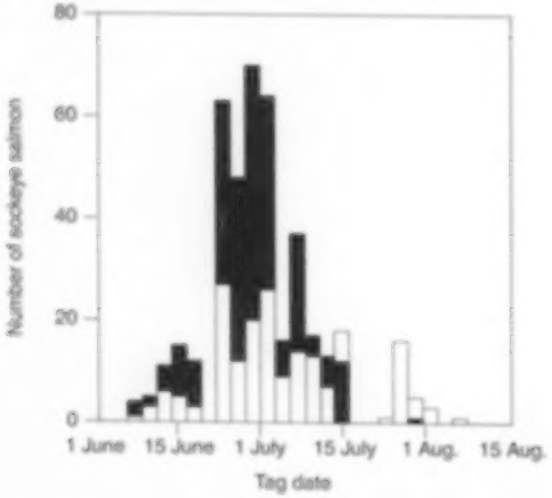
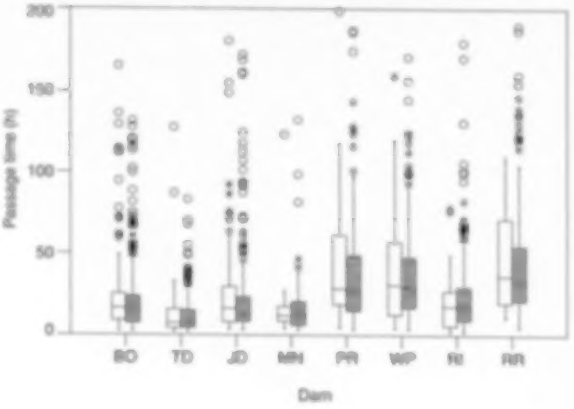


Fig. 10). Salmon ascended the unimpounded Hanford Reach at a slower rate (mean = 26.9 km·day^{-1}) than they did reservoirs (Table 7; Fig. 9). Radio-tagged sockeye salmon arrived at the lower Hanford receivers and at receivers at the upper ends of reservoirs throughout the day and night in 1997 (Fig. 8), suggesting frequent movement within reservoirs at night. In contrast, first records at the upper end of the Hanford Reach were more frequent during daylight hours.

Fig. 5. Passage times at each dam for successful (shaded boxes) and unsuccessful (open boxes) migrating sockeye salmon. Passage times >200 h are excluded for clarity: five fish for Bonneville (216–402 h), two fish for John Day (294 and 316 h), six fish for Priest Rapids (231–448 h), four fish for Wanapum (216–402 h), three fish for Rock Island (207–243 h), and one fish for Rocky Reach (344 h). Dam abbreviations: BO, Bonneville; TD, The Dalles; JD, John Day; MN, McNary; PR, Priest Rapids; WP, Wanapum; RI, Rock Island; RR, Rocky Reach. Asterisks (*) represent outliers and circles represent extreme outliers.



Hydrosystem passage times

Passage times and rates through longer hydrosystem reaches integrated multiple dam and reservoir passages and time fish spent falling back over and reascending dams. Passage times for 189 sockeye salmon from the Bonneville Dam tailrace to pass McNary Dam (four dams, three reservoirs, 238 km) ranged from 4.0 days (59.5 km·day^{-1}) to more than 29 days (8.2 km·day^{-1}) with a median time of 6.8 days (34.5 km·day^{-1}). Passage times for 178 sockeye salmon from Bonneville Dam past Priest Rapids Dam (five dams, four reservoirs, Hanford Reach, 407 km) ranged from 8.5 days (47.9 km·day^{-1}) to more than 30 days (13.6 km·day^{-1}) (median = 12.9 days, 31.5 km·day^{-1}). Median passage times were 17.3 days (28.7 km·day^{-1}) for 397 sockeye salmon from the Bonneville Dam tailrace past Rock Island Dam (seven dams, six reservoirs, Hanford Reach, 497 km) and 19.0 days (27.9 km·day^{-1}) for 212 fish from Bonneville Dam past Rocky Reach Dam (eight dams, seven reservoirs, Hanford Reach, 530 km).

Salmon with complete migration records spent similar proportions of time passing dams and ascending reservoirs (dams: 36.6%, 31.3%–41.9%; reservoirs: 43.1%, 38.2%–48%; mean and 95% confidence interval) (Fig. 11). Salmon spent nearly one fifth of their total migration time ascending the Hanford Reach (17.9%, 15.7%–20.2%; Friedman test $F_{\mathrm{F}} = 32.80$, df = 3, $p < 0.001$, $n = 12$), nearly identical to the proportion of the total monitored migration distance that this river section represents (17.1%). Other activities such as time spent falling back over and reascending dams accounted for a small proportion of time (2.4%, 0%–5.1%).

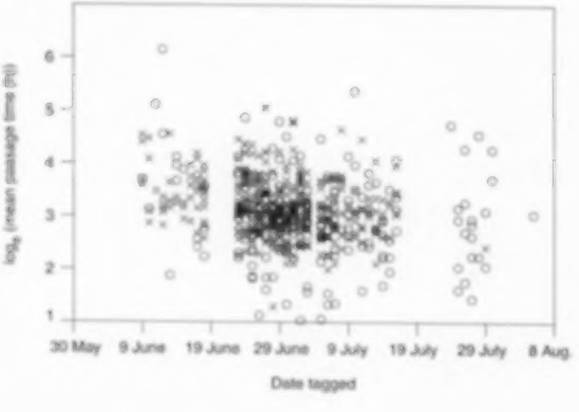
Table 5. Results of repeated-measures ANOVA testing for differences in $\log_e$(mean passage time) at dams and contrasts testing for differences between lower versus mid-Columbia River dams and among dams within river region.

Effect	Numerator df	Denominator df	*F*	*p*
Fate	1	551	4.65	0.0314
Dam	7	1933	52.25	<0.0001
F1 hour[a]	23	1735	2.47	0.0001
Tag date	1	551	44.96	<0.0001
Dam × fate	7	1933	1.75	0.0934
Contrast				
LC versus MC	1	1933	163.32	<0.0001
BO versus TD	1	1933	86.08	<0.0001
BO versus JD	1	1933	0.41	
BO versus MN	1	1933	5.31	
TD versus JD	1	1933	82.64	<0.0001
TD versus MN	1	1933	10.7	
JD versus MN	1	1933	7.29	
PR versus WP	1	1933	1.53	
PR versus RI	1	1933	47.43	<0.0001
PR versus RR	1	1933	0.04	
WP versus RI	1	1933	41.86	
WP versus RR	1	1933	1.79	
RI versus RR	1	1933	46.26	<0.0001

Note: Dam abbreviations: LC, lower Columbia River dams; MC, mid-Columbia River dams; BO, Bonneville; TD, The Dalles; JD, John Day; MN, McNary; PR, Priest Rapids; WP, Wanapum; RI, Rock Island; RR, Rocky Reach.

[a] F1 hour is the hour that sockeye salmon first arrived at the dam tailrace.

Fig. 6. Passage time at all dams in relation to date of tagging at Bonneville Dam for successful (crosses) and unsuccessful migrants (circles). The median untransformed passage time was 17.1 h (range 2.0–480.0 h).



Discussion

During the high-water year of 1997, approximately two thirds of tagged sockeye salmon successfully migrated past seven or nine dams in the Columbia River hydrosystem to reach spawning tributaries. Most striking was the decline in survival during the late portion of the run as flows decreased and temperatures increased. Notably, migration success was related to overall migration speed — unsuccessful migrants exhibited slow passage of dams and reservoirs, although this effect appeared to be scale dependent because the relationship between passage time and fate was not significant at most dams. General examination of migration revealed that migration was faster in the lower Columbia and quickened through the migration season. Dam passage and migration through the Hanford Reach were slower and displayed greater diel periodicity compared with migration in reservoirs. Below, we discuss potential mechanisms that could generate these patterns and implications for management, first at the hydrosystem scale and then at the finer scale of individual reaches.

Patterns of migratory timing and survival

The majority of radio-tagged sockeye salmon reached spawning tributaries and survival estimates were comparable with those reported for other Columbia River salmonids (Dauble and Mueller 2000). Dam counts are the primary method of estimating survival of adult salmon between hydroelectric projects, although several potential sources of error contribute to uncertainty in survival estimates (Dauble and Mueller 2000). To our knowledge, there have been no direct estimates of system-wide survival for individual Columbia River sockeye salmon runs prior to this study. We believe that survival estimates derived from telemetry studies are more reliable than those calculated from dam counts or passive integrated transponder tagging because they track individual fish, account for fallback, can better partition known and unaccounted-for loss factors including straying and fisheries take, and provide greater resolution to detect patterns such as the seasonal decline in survival observed here.

Table 6. Results of Cox proportional hazards regression test of passage hazard at each dam.

Dam	Variable	df	Parameter estimate	SE	χ^2	p	Hazard ratio
Bonneville (event = 502, censored = 52)	Fate	1	0.2679	0.1049	6.5234	0.0106	1.3070
	Time of day	1	–2.3530	0.1908	152.0245	<0.0001	0.0950
	Passage date	1	–0.0001	0.0044	0.0006	0.9813	1.0000
	Fork length	1	0.0092	0.0155	0.3535	0.5521	1.0090
	Sex	1	0.0495	0.1004	0.2436	0.6216	1.0510
The Dalles (event = 414, censored = 5)	Fate	1	0.1489	0.1326	1.2610	0.2615	1.1610
	Time of day	1	–1.4488	0.1537	88.8311	<0.0001	0.2350
	Passage date	1	0.0060	0.0052	1.3517	0.2450	1.0060
	Fork length	1	–0.0038	0.0164	0.0533	0.8174	0.9960
	Sex	1	0.1405	0.1137	1.5269	0.2166	1.1510
John Day (event = 341, censored = 26)	Fate	1	0.2685	0.1495	3.2247	0.0725	1.3080
	Time of day	1	–1.9914	0.2266	77.2051	<0.0001	0.1370
	Passage date	1	0.0185	0.0061	9.2286	0.0024	1.0190
	Fork length	1	0.0065	0.0169	0.1489	0.6996	1.0070
	Sex	1	–0.0164	0.1214	0.0182	0.8926	0.9840
McNary (event = 141, censored = 419)	Fate	1	0.1932	0.2467	0.6135	0.4335	1.2130
	Time of day	1	–2.3802	0.3693	41.5426	<0.0001	0.0930
	Passage date	1	–0.0348	0.0132	6.9467	0.0084	0.9660
	Fork length	1	–0.0741	0.0311	5.6826	0.0171	0.9290
	Sex	1	–0.1968	0.1903	1.0695	0.3011	0.8210
Priest Rapids (event = 175, censored = 255)	Fate	1	0.1501	0.2377	0.3989	0.5277	1.1620
	Time of day	1	–1.2425	0.2154	33.2671	<0.0001	0.2890
	Passage date	1	–0.0221	0.0104	4.5761	0.0324	0.9780
	Fork length	1	–0.0145	0.0270	0.2898	0.5904	0.9860
	Sex	1	0.1201	0.1653	0.5273	0.4677	1.1280
Wanapum (event = 381, censored = 12)	Fate	1	–0.0062	0.1846	0.0011	0.9734	0.9940
	Time of day	1	–1.4565	0.1659	77.0916	<0.0001	0.2330
	Passage date	1	–0.0312	0.0072	19.0141	<0.0001	0.9690
	Fork length	1	–0.0402	0.0160	6.3131	0.0120	0.9610
	Sex	1	0.0406	0.1134	0.1283	0.7202	1.0410
Rock Island (event = 371, censored = 17)	Fate	1	–0.1465	0.2119	0.4778	0.4894	0.8640
	Time of day	1	–1.1790	0.1390	71.9670	<0.0001	0.3080
	Passage date	1	0.0030	0.0073	0.1727	0.6777	1.0030
	Fork length	1	–0.0438	0.0179	5.9897	0.0144	0.9570
	Sex	1	0.2453	0.1163	4.4497	0.0349	1.2780
Rocky Reach (event = 206, censored = 21)	Fate	1	0.1022	0.2430	0.1769	0.6741	1.1080
	Time of day	1	–1.5761	0.2227	50.0677	<0.0001	0.2070
	Passage date	1	–0.0641	0.0112	32.6743	<0.0001	0.9380
	Fork length	1	–0.0616	0.0299	4.2380	0.0395	0.9400
	Sex	1	0.1089	0.1603	0.4613	0.4970	1.1150

Individual sockeye salmon migration success was strongly related to arrival date at Bonneville Dam. We used the date of tagging as a summary measures because river discharge, spill levels at dams, river temperature, and date were all intercorrelated, preventing us from partitioning patterns in survival to these potential causes in a rigorous manner. Nonetheless, temperature was probably the primary factor affecting migration success. Elevated water temperatures during upstream migration have been linked to higher in-river and prespawning mortality of sockeye salmon in the Columbia Basin (Major and Mighell 1967) and the Fraser River (Gilhousen 1990; Macdonald et al. 2000; Cooke et al. 2004). Adult exposure to elevated temperatures can increase susceptibility to disease and compromise reproductive performance (Coutant 1999; Torgersen et al. 1999) through increased metabolic demands (Rand and Hinch 1998), reduced allocation to gonadal development (Kinnison et al. 2001), and reduced egg viability (Berman and Quinn 1991). Extended exposure of adult salmonids to water temperatures >18 °C may increase the risk of prespawning mortality (mortality after adults have reached spawning tributaries; Becker and Fujihara 1978; Gilhousen 1990), and temperatures above 24 °C are lethal (Servizi and Jensen 1977). Cooke et al. (2004) identified several mechanisms that may have contributed to the dramatic in-river and prespawn mortality observed in late-run sockeye salmon in the Fraser River since 1995. While underlying causes of mortality are still unclear in the Fraser River, it is likely that temperature plays a direct or indirect role because the high mortality has been observed in a portion of the run entering the river more

Fig. 7. Passage time of sockeye salmon at individual dams in relation to the hour of first detection at the tailrace receiver. Observations are blocked by the number of nights elapsed between the first detection and subsequent passage. Numbers of nights at individual dams are zero (○), one (×), two (+), three (△), and four (▽). Passage times were longer for salmon arriving under cover of night compared with those arriving during the day, within nights at dam.

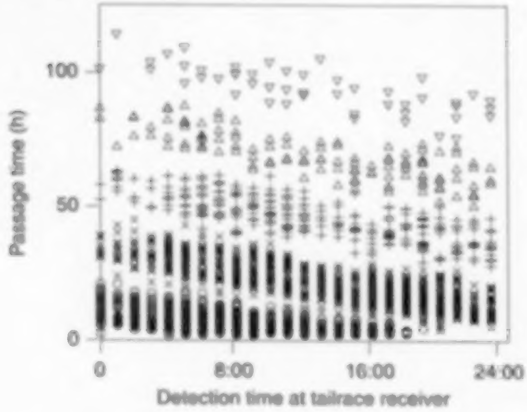


Fig. 8. (*a*) Diel pattern of records at the upper end of reservoirs and base of dams, (*b*) time of exit from top-of-ladder, (*c*) time at the downstream end of Hanford Reach, and (*d*) time at the upstream end of the Hanford Reach.

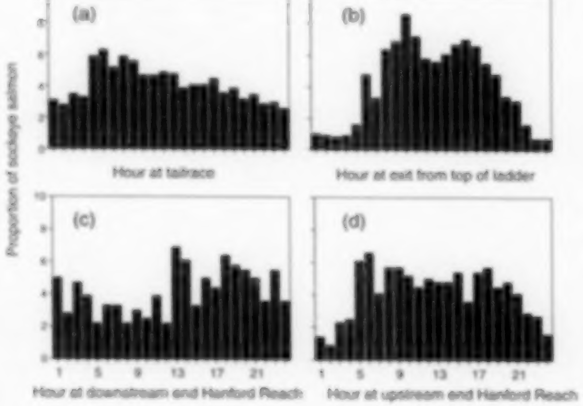
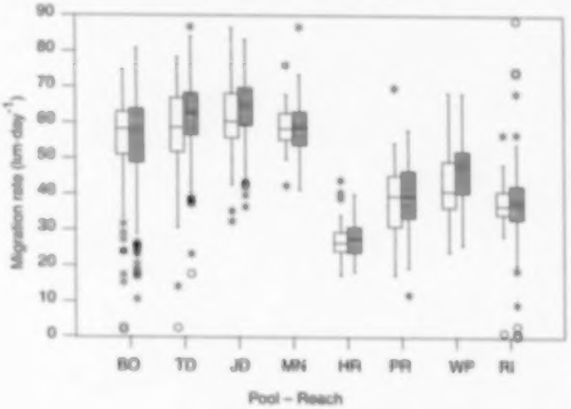


Fig. 9. Migration rate through reservoirs and Hanford Reach in relation to eventual fate of each sockeye salmon. Open boxes represent unsuccessful migrants and shaded boxes represent successful migrants. Pool and reach abbreviations: BO, Bonneville; TD, The Dalles; JD, John Day; MN, McNary; HR, Hanford Reach; PR, Priest Rapids; WP, Wanapum; RI, Rock Island. Asterisks (*) represent outliers and circles represent extreme outliers.



than 6 weeks earlier in the season than the historical pattern, shifting migration to a period of warmer water temperatures in August (Cooke et al. 2004). In our study, the precipitous drop in migration success occurred for sockeye salmon tagged after the second week of July as temperatures at Bonneville Dam and elsewhere in the drainage exceeded 20 °C.

The pattern of mortality that we observed could have been caused by other measured or unmeasured factors or may have arisen through a temperature-dependent tagging effect. Unfortunately, we could not determine the relationship between temperature and any handling effects with our study design, although several lines of evidence suggest a minimum handling effect. First, there was no mortality during tagging, transport, or release. Second, while both sources of mortality would contribute to the decline in migration success late in the season, telemetry data indicated that the tagged fish in the late period did not die immediately after release, with most fish passing two or more dams and living a week or more post-tagging during the late period. While both sources of mortality would produce a similar pattern of migration success, these data indicate that the tagged fish in the late period did not die immediately after release. Finally, we estimated survival for sockeye salmon migrating during these two periods using dam count data from Bonneville and Rock Island dams and assuming a 16-day passage time between these dams (the mean migration time observed for sockeye salmon tagged on 4–16 July). Although these estimates have several potential sources of error, a large decline in survival was also observed in the dam count sample, with estimated migration success dropping from 94.2% to 56.3% later in the summer. Taken together, ancillary evidence suggests that tag and handling effects were not the primary cause of the decline in survival that we observed. In addition, the seasonal pattern of mortality could not be attributed to changes in seasonal fishing pressure: only 2 of 65 fish reported captured in tribal fisheries were tagged during the late period.

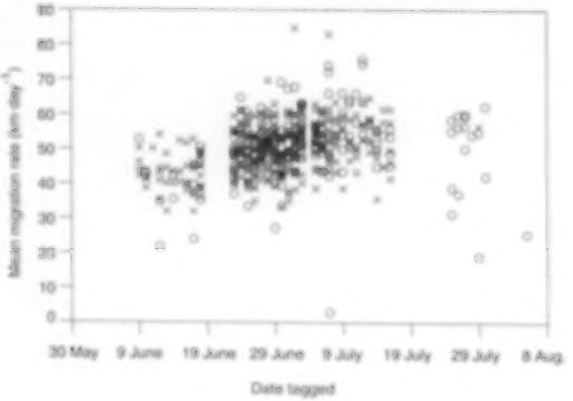
Several mechanisms may have contributed to the observed relationships among migration success, speed, and season. First, the relationship between migration success and speed could be direct, where slowed migration caused by dams resulted in the expenditure of energy that slowed migration at upstream projects and subsequently lowered the probability of successful migration. Alternatively, slow migration may have resulted from poor initial physiological condition leading to slowed migration, increased thermal exposure, and subsequent low probability of migration success. These two mechanisms are not mutually exclusive and rather would likely work synergistically to decrease migration success.

Table 7. Results of repeated-measures ANOVA and contrasts for the effects of season on migration rates among reaches.

Effect	Numerator df	Denominator df	F	p
Fate	1	492	20.87	<0.0001
Dam	7	1607	274.41	<0.0001
Tag date	1	492	81.96	<0.0001
Dam × fate	7	1607	1.71	0.0992
Contrast				
LC (n = 3) versus MC (n = 4)	1	1607	686.86	<0.0001
Hanford Reach versus all reaches	1	1607	1047.65	<0.0001
BO versus TD	1	1607	30.52	<0.0001
BO versus JD	1	1607	75.04	<0.0001
BO versus MN	1	1607	6.44	0.0113
TD versus JD	1	1607	15.49	<0.0001
TD versus MN	1	1607	1.17	0.2789
JD versus MN	1	1607	14.75	0.0001
PR versus WP	1	1607	26.22	<0.0001
PR versus RI	1	1607	3.91	0.0482
WP versus RI	1	1607	63.54	<0.0001

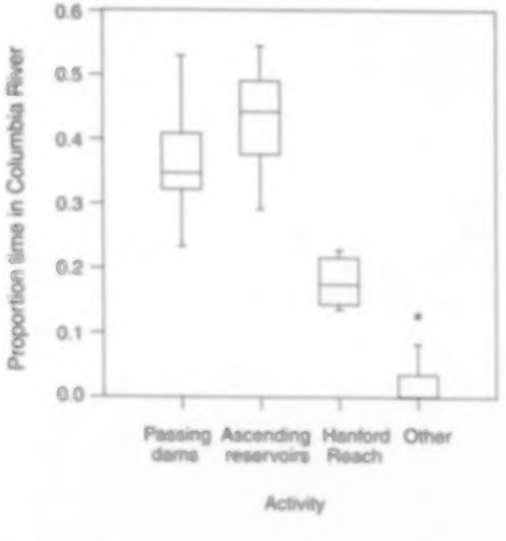
Note: Reach abbreviations: LC, lower Columbia River dams; MC, mid-Columbia River dams; BO, Bonneville; TD, The Dalles; JD, John Day; MN, McNary; PR, Priest Rapids; WP, Wanapum; RI, Rock Island.

Fig. 10. Migration rate in relation to date of tagging for successful (crosses) and unsuccessful (circles) migrating sockeye salmon.



Notably, the seasonal pattern of mortality may have been related to both factors. It is plausible that late-entry fish were in relatively poor initial physiological state, perhaps having delayed entry to continue ocean feeding, and this subsequently led to poor initial condition, slow migration rates, increased thermal exposure, and low migration success. Clearly, understanding the relationship between initial condition and migration success is important in a management context because the relative importance of each mechanism will determine the effectiveness of management actions aimed at improving passage conditions at dams — efforts to improve passage at dams will provide little benefit if migration success is primarily related to fish condition at river entry.

Fig. 11. Proportion of total migration time from the Bonneville Dam tailrace to the tailrace at Rock Island Dam that sockeye salmon recorded during first passages over dams, ascending reservoirs, ascending the Hanford Reach, or other activities including second passage times for salmon that fell back at dams. Analysis was limited to fish with complete passage records at five or six of the first six dams and in the Hanford reach (n = 90, including six fish that were not recorded in one of the spawning tributaries). Asterisk represents outlier.



Regardless of mechanism, the observed pattern of seasonal mortality suggests the potential for current selection on run timing. The upstream migration of anadromous salmonids is an energetically demanding part of the life cycle and its initiation is largely governed by the interactions of water temperature, flow regime, and other factors that influence maturation (Gilhousen 1990; Hodgson and Quinn 2002). Substantial variation exists in the timing of spawning migrations of North American sockeye salmon populations and is thought to relate to temporal variation in both migration conditions for adults and spawning and rearing conditions in tributaries (Tagaki and Smith 1973; Merritt and Roberson 1986; Hodgson and Quinn 2002). For example, Columbia River and interior British Columbia stocks of sockeye salmon tend to enter fresh water before peak summer temperatures and hold in spawning tributaries for 1 month or more before fall spawning whereas coastal stocks migrate after peak temperatures just prior to spawning (Gilhousen 1990; Hodgson and Quinn 2002). Quinn and colleagues (Quinn and Adams 1996; Quinn et al. 1997; Hodgson and Quinn 2002) have used dam counts and historical records to examine the relationships between run timing and environmental conditions. They found that sockeye salmon passed Bonneville Dam progressively earlier over the period 1949–1993, concomitant with a progressive increase in the mean temperatures that migrating adults experienced in the lower Columbia River (Quinn and Adams 1996). Patterns of individual mortality during 1997 in relation to run timing and temperature were consistent with selection for earlier run timing within these populations, as hypothesized by Quinn and Adams (1996).

If such selection is real and leads to evolution of earlier run timing, we speculate that the energetic costs of migration will increase in coming decades. Recent regional climate projections predict increasingly early winter snowmelt, longer dry periods during summer, and later onset of fall conditions (Parson et al. 2001). If true, these conditions would lead to continued selection for early run timing in Columbia River sockeye and other spring-run salmonids. The energetic costs of migration will likely increase because fish will either hold without feeding in spawning tributaries for longer periods or experience higher temperatures during migration if populations do not respond to selection as rapidly as conditions change or both. This increase in energetic cost could lead to greater in-river or prespawn mortality, particularly in warm years and for stocks migrating long distances.

Scale dependence in patterns of migration behavior and success

Our primary objective was to characterize migration behavior in the hydrosystem and determine if migration behaviors were related to migration success. The relationships between dam passage time and migration rates and migration success were significant when assessed through the lower Columbia River or for all of the migration history, but the relationship between passage time and migration success was not significant when analyzed at individual dams, with the exception of Bonneville Dam. This incongruity suggests that consistently slow passage through multiple projects was associated with unsuccessful migration, but passage times at individual dams by migrants of both types were variable and not significantly different between successful and unsuccessful migrants. The lack of a significant relationship between passage hazard and migration success probably did not result from a lack of statistical power, given our relatively large samples sizes. The scale dependence observed in the effect of migration behavior on migration success illustrates the importance of using fitness-based measures (e.g., survival to spawning tributaries) of performance and examining migration performance throughout the hydrosystem rather than performance at individual locations. Notably, our estimates only include in-river mortality. Prespawn mortality can be high in sockeye salmon populations and we were unable to assess any effects of migration history on prespawn mortality (Gilhousen 1990; Cooke et al. 2004). Therefore, we may have underestimated the effects of migration history on total adult survival.

In summary, these data suggest the potential for an indirect negative hydrosystem effect on migration success in sockeye salmon through the hydrosystem's influence on water temperature. Currently, the relative importance of hydrosystem alterations (i.e., dams) compared with changes in regional climate and land use in contributing to summer warming of the Columbia River is unknown. Nonetheless, we expect the observed pattern of late season mortality to become increasingly important if climate projections hold (Neitzel et al. 1991; Hamlet and Lettenmaier 1999; Parson et al. 2001). In contrast, we found little evidence for a direct effect of individual dams on adult passage in sockeye salmon.

General patterns in migration behavior

We also used telemetry data to examine general patterns of migration behavior throughout the hydrosystem, focusing on patterns of straying, migration over dams, and migration through reaches between dams. Accurate estimates of straying are important for effective management of salmon. In particular, straying and spawning in nonnatal tributaries can affect escapement estimates (Dauble and Mueller 2000). Straying behavior by salmonids occurs naturally and is important for populations because it leads to colonization of new habitats (Milner and Bailey 1989) and avoidance of unfavorable conditions (Leider 1989) and increases genetic diversity (Utter 1991). Sockeye salmon are noted for homing with high precision to their natal streams (Foerster 1936, 1968). In our study, 1.7% (10 of 574) of the radio-tagged sockeye salmon strayed to nonspawning tributaries, similar to the 1.0% stray rate reported by Quinn et al. (1987) for southern British Columbia rivers. Hence, survival estimates from dam counts may be less biased for sockeye than for other salmon species that stray at higher rates (Boggs et al. 2004). Alternatively, the fish that that we considered strays may have been searching rather than straying in the true sense (Griffith et al. 1999). Burger et al. (1995) found that 16% of radio-tagged sockeye salmon temporarily entered a different tributary than the one where they eventually spawned. However, undetected temporary straying was unlikely in the lower Columbia, where 9 out of 10 strays were last recorded, given the extent of telemetry coverage.

Most radio-tagged sockeye salmon passed Columbia River dams rapidly, <24 h after passing tailrace receivers. Median passage time between Rock Island and Rocky Reach dams in our study was 20.7 h compared with a modal time of 53 h reported by Major and Mighell (1967). At each dam, however, 0.2%–8% took more than 5 days to pass, and all time-to-pass distributions were right-skewed. Sockeye salmon passage times were similar to those for summer chinook salmon (*Oncorhynchus tshawytscha*) (medians 12–26.4 h at lower Columbia dams) whose run timing is similar to that of sockeye. In contrast, Columbia River sockeye salmon passed lower Columbia River dams much more quickly than most radio-tagged spring chinook salmon, which had median times between 16.8 and 67.2 h (30% took more than 5 days to pass in 1997; Keefer et al. 2004c). Differences in passage time for spring chinook versus summer chinook and sockeye salmon may be related to river conditions. Most spring chinook salmon migrated when flows were relatively high and river temperatures were cool compared with those encountered by sockeye later in the year. Fish may be able to find routes past dams more easily when flow and spill are low, but these effects appear to be relatively weak given the high variability in passage time.

Light level appeared to have the strongest effect on passage time. Specifically, few fish arriving in the tailrace late in the afternoon ascended before spending a night at the dam, consequently increasing passage times. These observations are consistent with previous observations in sockeye salmon (Quinn and Adams 1996) and steelhead (Robards and Quinn 2002) in the Columbia River. Low visibility may make navigation past the relatively complex barriers presented by dams and fishways too difficult for nighttime passage. High turbidity may have further induced daytime passage of dams by sockeye salmon. Secchi disk depths at Bonneville Dam in 1997 were approximately 50% less than the previous 10-year average during the time when radio-tagged sockeye salmon were passing Bonneville Dam, and this high turbidity was presumably caused by the high discharge conditions. In contrast, Hellawell et al. (1974) found that in clear water, most upstream movement of Atlantic salmon (*Salmo salar*) occurred in darkness, suggesting species differences in light sensitivity, complexity of river passage conditions for Atlantic salmon versus Pacific salmon in dam passageways, or both.

Migration rates through the lower Columbia River reservoirs observed here (57–65 km·day^{-1}) are among the highest daily migration rates reported for sockeye salmon. However, the rates are for single reservoir passage, and the median migration rate from Bonneville to McNary, incorporating dam passage times, for our sample was 33.8 km·day^{-1}, in the middle of the values of 22–49 km·day^{-1} reported by Quinn et al. (1997) based on dam counts for this same reach for the period 1954–1994. Migration rates were faster in the lower Columbia River than in the mid-Columbia River, and fish tagged later in the run migrated more quickly in the mid-Columbia River compared with fish tagged earlier in the run. Increasing migration speed for later arriving fish may be related to elevated metabolic activity as temperatures increased, discharge and turbidity decreased, and increased motivation as runs progressed and spawning time approached (Gard 1973; Hellawell et al. 1974; Gilhousen 1990). However, late-arriving fish migrated at a slower rate than those arriving during the peak of migration, again suggesting a potential effect of stressful temperatures on late migrants. Many sockeye salmon were recorded passing upstream in reservoirs and the lower end of the Hanford Reach (the top of the McNary Reservoir) during darkness; nighttime passage through these less heterogeneous environments may explain in part the rapid migration rates in impounded reaches relative to the unimpounded Hanford Reach.

Sockeye salmon migrated through the unimpounded section of the Columbia River (Hanford Reach) at slower rates than reported for several populations of Fraser River sockeye salmon (Killick 1955) and at about half the rate calculated for Columbia River impoundments. Rates for the Hanford Reach in this study were higher than those of sockeye salmon in the Karluk (Gard 1973) and Copper rivers (Merritt and Roberson 1986) and of chinook salmon in the Kenai River (Bernard et al. 1999) but lower than those of chinook salmon and fall-run steelhead in the Hanford Reach in 1997 (Keefer et al. 2004*b*). Slower passage through the Hanford Reach by sockeye salmon, as compared with Columbia River chinook salmon or steelhead, may be related to smaller body size (Brett 1965; Weihs 1973). In comparison with reservoirs, the higher velocities in the Hanford Reach were probably the major contributor to slower migration rates than were observed in the impounded reaches, and it is probable that energy expenditure in this reach was greater than that in reservoirs, despite lower ground speeds. For instance, Merritt and Roberson (1986) found that increased river level significantly decreased travel rate of sockeye salmon within a given year and between years. Notably, the observed rates through the Hanford Reach may underestimate the long-term average because of the high discharge conditions of 1997.

Current and historical estimates of migration rate

The telemetry data allowed us to calculate time budgets for individual fish during migration. Interestingly, the time spent passing the Hanford Reach was proportional to the total migration distance monitored. By extension, the proportion of time spent passing dams and ascending reservoirs was proportional to the distance traveled, suggesting that rapid passage of reservoirs compensated for slowed migration at dams, resulting in similar overall migration rates in the impounded and unimpounded portions of the hydrosystem.

River conditions encountered by migrating Columbia River salmonids have been dramatically altered and the effect of dams on migration times is unknown because no predam data are available. Salmon in unimpounded rivers frequently encounter migration obstacles, such as falls, rapids, or channel constrictions, and dams may not effectively differ from other obstructions. For example, migration speeds for Fraser River sockeye salmon are lowest through constricted and high-velocity areas (Gilhousen 1990; Hinch et al. 1996; Hinch and Rand 1998) and Atlantic salmon migrations are temporarily blocked at falls in Norway (Jensen et al. 1989).

Predam passage rates in the mainstem Columbia River can be estimated from rates for fish in the unimpounded Hanford Reach assuming that passage conditions in this reach are representative of historical conditions throughout the river. Using the rate for sockeye salmon in the Hanford Reach, we estimate that predam Columbia River passage times from river kilometre 235 (Bonneville Dam) to river kilometre 730 (Rock Island Dam) would have been 17.6 days compared with the 17.3 days that we measured in 1997. Similar results were found for spring and summer chinook salmon by applying rates from the unimpounded Snake and Salmon rivers (Keefer et al. 2004*c*). They estimated that predam Columbia and Snake River passage times from river kilometre 235 (Bonneville Dam) to river kilometre 695 (Lower Granite Dam) would have been slightly longer for spring chinook and slightly shorter for summer chinook salmon without the dams. There are clear limitations to such estimates, but they further suggest that rapid migration through reservoirs by adults may largely compensate for slowed migration at dams.

While we found no evidence for negative effects of the Columbia River hydrosystem on adult sockeye salmon passage, inferences based on these data should be made cautiously for at least two reasons. First, because we were unable to assess reproductive success, we do not know how reproductive success is related to migration behavior in the hydrosystem, and any negative indirect effects of dam passage would have gone undetected (e.g., increased prespawning mortality after entering spawning tributaries in sockeye salmon with relatively long passage times). Second, because data were available for only a single (unusual) year, how migration success varies under different environmental conditions remains unknown. Thus, we caution that pronounced direct effects of dam passage may exist, particularly in low-water, high-temperature years. Clearly, more data are needed, both in the Columbia River and in similar rivers without impoundments such as the Fraser River, before a robust picture of sockeye salmon migration and the influence of dams on adult passage is obtained.

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Comparing naturally occurring stable isotopes of nitrogen, carbon, and strontium as markers for the rearing locations of Atlantic salmon (*Salmo salar*)

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Abstract: We compared the success of using naturally occurring stable isotopes of N, C, and Sr as markers for the rearing locations of juvenile salmon. We analyzed the isotopic signatures (δ^{15}N and δ^{13}C in muscle and scales and ^{87}Sr/^{86}Sr in otoliths) of >200 juvenile Atlantic salmon (*Salmo salar*) from 12 tributaries of the Connecticut River, USA. Young salmon had distinct N and C signatures 5 weeks after stocking. Signatures were stable over the summer although δ^{13}C varied more than δ^{15}N or ^{87}Sr/^{86}Sr. Scale and muscle signatures were highly correlated, demonstrating the feasibility of nonlethal sampling using fish scales. Some C (but not N) signature from the hatchery was retained in scales of 3-month-old fish, implicating scale annuli as a repository for past C signatures. The δ^{15}N values successfully differentiated fish from tributaries with differences in land use (e.g., agricultural versus forested; ≈33% of sites); the δ^{13}C values differentiated fish from 45% of sites. Based upon a discriminant-function analysis, group membership of individuals was correctly predicted in 44.3% (74 of 167) of cases for which both N and C were analyzed. In combination, N and C isotopes differentiated 73% of study sites, which was close to the success of Sr isotopes in the same system (83%).

Résumé : Nous avons comparé la performance des isotopes stables de N, C et Sr qui existent en nature comme marqueurs du lieu où se sont développés des jeunes saumons. Nous avons analysé les signatures isotopiques (δ^{15}N et δ^{13}C dans le muscle et les écailles et ^{87}Sr/^{86}Sr dans les otolithes) chez >200 jeunes saumons atlantiques (*Salmo salar*) dans 12 tributaires du fleuve Connecticut, États-Unis. Les jeunes saumons possèdent des signatures N et C distinctes 5 semaines après l'empoissonnement. Les signatures demeurent stables au cours de l'été bien que δ^{13}C varie plus que δ^{15}N ou que ^{87}Sr/^{86}Sr. Il y a une forte corrélation entre les signatures du muscle et des écailles; il est donc possible de faire des prélèvements non létaux au moyen des écailles des poissons. Des vestiges de la signature de C (mais non de N) de la pisciculture persistent dans les écailles des poissons âgés de 3 mois; les annulus des écailles sont donc des points de rétentions des signatures antérieures de C. Les valeurs de δ^{15}N permettent de séparer avec succès des tributaires dans des zones d'utilisation des terres différentes (par exemple, les zones agricoles des zones forestières : ≈33 % des sites); δ^{13}C permet la reconnaissance de 45 % des sites. Une analyse des fonctions discriminantes permet de prédire correctement l'appartenance des individus à un groupe dans 44,3 % (74/167) des cas lorsque C et N ont été tous deux analysés. La combinaison des isotopes N et C permet de séparer 73 % des sites, ce qui s'approche de la performance des isotopes Sr dans le même système (83 %).

[Traduit par la Rédaction]

Introduction

Geochemical signatures are increasingly used to identify geographic sources, habitat use, and movements of animals (Campana and Gagne 1995; Kennedy et al. 1997; Thorrold et al. 1998). Animals preserve chemical records from their environment in their tissues. As a result, in aquatic systems predictable relationships arise between underlying geology, land use, water chemistry, food-web structure, and tissue chemistry of residents of particular habitats (Limburg 1995;

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Harrington et al. 1998; Thorrold et al. 2001). Efforts to link fish to specific geographic locations and to supplement conventional fish-tagging techniques using unique geochemical markers have been compelling and productive.

Stable isotopes offer numerous advantages over other stock-identification techniques for identifying rearing locations (for a review see Hobson 1999). Isotopic signatures occur naturally and therefore require no invasive handling or application cost. Techniques for measuring isotope ratios are precise and steadily improving. Isotope ratios can be measured in fish of all ages and sizes and so are especially useful in larval fish, which are too small for conventional tags. Finally, isotopic signatures in fish (France 1995*b*; Kennedy et al. 1997; Wells et al. 2000*b*) and other animals (Marra et al. 1998; Blum et al. 2001; Rubenstein et al. 2002) correlate highly with environmental conditions in their rearing habitats.

Isotopic signatures that originate in underlying bedrock (e.g., Sr isotopes) are particularly useful because they remain fairly constant seasonally and annually (Kennedy et al. 2000; Peters et al. 2004). Consequently, Sr isotope signatures are stable as long as a fish remains in a particular site. Yet no single isotope can discriminate among an infinite number of source locations, so the isotopic signatures of multiple elements are often used to greatly enhance discriminatory power (Koch et al. 1995; Doucett et al. 1999). In any particular study, the choice of suitable elements or isotopes for providing habitat signatures will depend upon considerations of both precision and price.

Many different elemental (e.g., Mg, Ba, Sr, Mn relative to Ca) and isotope ratios (e.g., $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $^{87}Sr/^{86}Sr$) have been used to trace the origins of moving and migrating fish (Doucett et al. 1999; Hobson 1999; Thorrold et al. 2001). Sr has been among the most successful because of its relative abundance in nature and because it provides both an isotopic and an elemental fingerprint with high precision (Kennedy et al. 2000, 2002). Sr is incorporated into the growing tissue of fish through substitution for Ca. Sr signatures in their calcified tissues mirror Sr signatures in the water in which they are reared (Kennedy et al. 2000). Large differences between freshwater and marine Sr concentrations enable the separation of anadromous from non-anadromous fish stocks based upon the elemental ratio of Sr to Ca in their bones and otoliths (Limburg 1995; Friedland et al. 1998; Radtke et al. 1998). In comparison, stable isotope signatures of Sr in fish reflect differences in the $^{87}Sr/^{86}Sr$ ratios that arise from relatively small differences in watershed geology (Kennedy et al. 1997; Ingram and Weber 1999; Kennedy et al. 2000). Thus, they can be used to distinguish fish habitat use over comparatively small spatial scales. A close match between the isotopic signature of a fish and that in a particular stream indicates that the fish could have reared in that habitat (Kennedy et al. 2002). Discrepancies between the isotopic signature of individuals and that of their stream-water indicate that there has been movement among streams (Kennedy et al. 2000, 2002). Sr isotopes have made it possible to distinguish fish from multiple tributaries within a catchment (Kennedy et al. 1997; Ingram and Weber 1999) and to resolve the movements of individual fish among rearing streams (Kennedy et al. 2000, 2002). This level of spatial resolution in freshwater systems (1–10 km) has not been possible using elemental ratios (e.g., Sr/Ca).

In this study, we quantitatively compare the relative success of naturally occurring stable isotopes of N, C, and Sr at distinguishing among rearing tributaries of juvenile Atlantic salmon (*Salmo salar*). A comparison of these isotopic systems is useful because most laboratories will have the capacity to measure some isotopic ratios but not others. Moreover, these isotopic systems each mark different aspects of the stream habitat and may vary over different temporal and spatial scales. For example, the $\delta^{15}N$ value of stream water is sensitive to differences in land-use patterns at the watershed scale because many agricultural processes result in enrichment of $\delta^{15}N$ relative to forested areas (McClelland et al. 1997; Harrington et al. 1998; Lake et al. 2001). The $\delta^{15}N$ value is also used to determine the trophic positions of fish and other animals (Deniro and Epstein 1978; Fry 1991; Cabana and Rasmussen 1994). In comparison, the $\delta^{13}C$ value of stream organisms varies seasonally and spatially as a function of the source of organic matter (e.g., benthic algal versus terrestrial C subsidies) or the longitudinal location within a watershed (Rounick and Winterbourn 1986; Finlay et al. 1999; Finlay 2001). Finally, the $^{87}Sr/^{86}Sr$ ratio of stream water is tightly linked to the underlying watershed geology (Kennedy et al. 2000), which is very stable over time. Sr isotopes also do not fractionate significantly within organisms, and any fractionation that might occur is removed from the isotopic signal during analysis (Kennedy et al. 2000). Hence, these isotopes investigated together are likely to provide a more powerful tool for stock discrimination than each does alone. Other researchers have investigated the use of isotopes or trace elements in combination to follow movements or origins of various species of fish (Hansson et al. 1997; Thorrold et al. 1998; Doucett et al. 1999), birds (Marra et al. 1998; Rubenstein et al. 2002), or invertebrates (DiBacco and Levin 2000). However, none compare the stable-isotope ratios of these three key elements or evaluate them at the spatial resolution of our study.

We measured the isotopic signatures ($\delta^{15}N$, $\delta^{13}C$) in muscle tissue and scales of >200 juvenile (age 0) Atlantic salmon stocked into 12 tributaries of the Connecticut River, USA, as part of the Atlantic salmon restoration program. We first compare the ability of $\delta^{15}N$ and $\delta^{13}C$, alone and in combination, to distinguish fish from different sites. We then compare $\delta^{15}N$ and $\delta^{13}C$ signatures relative to the power of $^{87}Sr/^{86}Sr$ ratios in fish from the same region, based upon our previous analyses (Kennedy et al. 2000). Specifically, we quantify (*i*) the rates at which N- and C-isotope signatures are incorporated into salmon fry after stocking, (*ii*) the stability of isotopic signatures in fish tissues within the first summer of growth, (*iii*) the relationships between the N- and C-isotope signatures in scales and muscle tissue (with particular interest in the feasibility of developing a method for nonlethal isotope assessment), and (*iv*) the ability of $\delta^{13}C$ and $\delta^{15}N$ to distinguish between fish from different sites and how the discriminatory power of these two isotopes compares with that of a geologically derived tracer, the $^{87}Sr/^{86}Sr$ ratio.

Methods

We collected juvenile Atlantic salmon from 12 stream tributaries of the West and White rivers in central and southern Vermont, USA (Fig. 1), for C- and N-isotope analysis. These streams are stocked annually in early May with unfed hatchery-raised fry (<30 mm). Currently 15% of the more than 10 million fry stocked annually in the Connecticut River restoration program are placed in the tributaries of the West and White rivers. We sampled the muscle tissue and scales of 200 juvenile salmon from four tributaries in the West River basin and eight sites in the White River basin in 1991, 1995, and 1996. We analyzed and previously reported ^{87}Sr/^{86}Sr ratios in salmon collected from West River and White River tributaries (Kennedy et al. 1997, 2000) and some of these results are included to compare the efficacy of discriminating populations based upon stable isotopes of N, C, and Sr separately and in combination.

Sample collection and preparation

We collected underyearling Atlantic salmon by means of a combination of electroshocking and dip-netting techniques (described in Nislow et al. 1999). Wet weights and lengths were taken in the field. Fish were immediately placed on ice and stored frozen at –20 °C. Prior to isotopic analysis, samples were thawed and dissected in the laboratory. Dissections were cleanly performed using acid-washed Teflon® forceps or clean stainless-steel tools. For consistency, a small sample of bone-free muscle tissue was taken from the left side of each fish posterior to the dorsal fin. Between 8 and 10 scales were removed from the front left side of the fish dorsal to the lateral line. Scales were first rinsed thoroughly with triple-deionized and distilled (3×) water, then any visible organic residue was carefully scraped off under a dissecting microscope. Scales were then rinsed with ultrapure dilute (0.1 mol·L^{-1}) NaOH to remove any additional residue, ultrasonically cleaned for 5 min in 3× water, and rinsed thoroughly with 3× water. Muscle tissue was rinsed thoroughly with 3× water. Muscle tissue and scales were dried overnight at 60 °C and ground to a fine powder in a Wiley Mill with a No. 40 sieve and subsamples were weighed in tin capsules. To retrieve enough material, multiple scales from individuals were combined for each single analysis.

Uptake and stability of N and C isotopes

To measure the rate of incorporation and temporal variability of N- and C-isotope signatures in salmon muscle tissue, we sampled juvenile salmon in six sites (Marlboro, Flood, Utley, Bethel Gilead, Tweed, and Hancock) up to three times during their first growing season in 1991. These periods corresponded to approximately 40–50, 60–75, and 80–107 days after stocking. Between 5 and 10 individuals were caught and analyzed for a given site and date. Hatchery fry, which were obtained prior to stocking in 1995 and 1996 from the White River National Fish Hatchery in Bethel, Vermont, USA, were pooled and used to give an initial isotopic signature. All tissues were prepared for N- and C-isotope analysis as described under "Analytical procedures".

Relationship between δ^{15}N and δ^{13}C signatures of salmon muscle tissue and scales

To compare isotopic signatures of different fish tissues, we compared δ^{15}N and δ^{13}C in scales and muscle tissue from salmon collected in six sites (5–8 fish per site) 4 months after stocking (in 1996). The six sites (White River – Peavine, First Branch, Third Branch, West Branch, Bingo Brook, and Bethel Gilead) were selected to span the land-use patterns that typify the relatively forested headwater streams in the region (see Harrington et al. 1998) and thus to produce a potential range of δ^{15}N and δ^{13}C signatures. Salmon from all sites were pooled in the analysis. We analyzed different individuals from these six sites previously for N in muscle tissue only (Harrington et al. 1998). However, a completely new data set for C and N in muscle tissue and scales is presented here.

Distinguishing among salmon populations

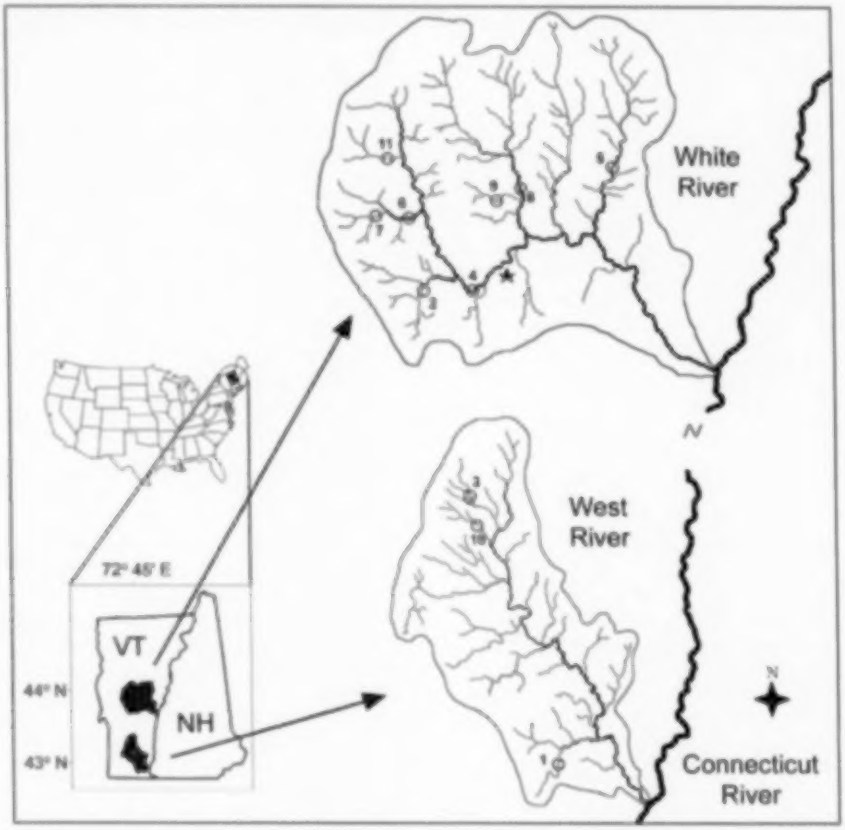
To characterize regional variation in δ^{15}N and δ^{13}C signatures across salmon streams, we tested for differences in isotopic signatures among fish from 12 study streams (11 sites for δ^{13}C because fish from 1 site were only analyzed for N). These are the streams referred to in the previous sections that were used to test for uptake, stability, and the relationship between signals in muscle and scales (Fig. 1). All fish taken from a site were used to establish a mean signature for each site. In cases where fish were collected at different times throughout the growing season, data were pooled over all sampling dates. We felt that pooling across the entire time period would ensure proper interspersion of samples across the growing season and the most conservative test of site differences, because variability in the isotopic signatures is larger when data are included for the entire time period than for any single time point. To test the effect of pooling all individuals over the entire growing season on our ability to discriminate sites, we compared the results of two discriminant-function analyses for late-season fish from the five sites for which three sampling dates are represented. First, we attempted to reclassify the 37 late-season fish based upon discriminant functions constructed when all 118 fish were included and then compared the results with discriminant functions based only on those 37 fish.

We compared the mean δ^{15}N and δ^{13}C values for fish across sites using one-way analysis of variance. Analysis of single variables was performed using one-way analysis of variance followed by Fisher's least significant difference multiple comparisons test. Fisher's least significant difference multiple comparison ($p < 0.05$) was used to test for significant differences in δ^{15}N and δ^{13}C signatures across sites. Sites were placed in distinct groupings based upon whether they had significantly different C- and (or) N-isotope signatures. We performed multivariate analysis on δ^{15}N and δ^{13}C signatures using Fisher's linear discriminant function analysis to compare our among-site comparisons with individual reclassification rates. SPSS® for Windows (version 11.5.0; SPSS Inc. 2002) was used for the statistical analysis.

Analytical procedures

N- and C-isotope compositions were determined using an online Carlo Erba elemental analyzer instrument interfaced with a continuous-flow Finnigan MAT 252 (Thermo Finnigan MAT, Bremen, Germany) mass spectrometer located at Dartmouth College, New Hampshire, USA. Isotope ratios for N and C are presented as δ values, where $\delta = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ and $R = {}^{15}\text{N}/{}^{14}\text{N}$ or ${}^{13}\text{C}/{}^{12}\text{C}$. The N- and C-isotope reference standards are air (N) and

Fig. 1. Map of the study sites in the White and West rivers, Vermont, USA. The star beside the White River indicates the location of the White River National Fish Hatchery, which provides most of the Atlantic salmon (*Salmo salar*) fry for the stocking program. Numbered symbols identify study sites listed in Table 2 and shown in Fig. 4.



Peedee belemnite (C). Reported uncertainties are the standard error (SE) of the mean.

For each batch of analyses (approximately 10 samples) we prepared and analyzed our laboratory standard $(NH_4)_2SO_4$ (Baker Analyzed, VWR). The laboratory standard had a $\delta^{15}N$ value of −1.20 ± 0.02‰ ((mean ± 1 SE; $n = 49$) for the duration of this experiment. We also analyzed N standards with $\delta^{15}N$ values determined by the Geophysical Laboratory of the Carnegie Institution. We determined a value of 7.19 ± 0.06‰ (mean ± 1 SE) for the Geophysical Laboratory's $NaNO_3$ standard, which has a value of 7.2‰ ($n = 8$). For gelatin with a Geophysical Laboratory value of 7.4‰ for $\delta^{15}N$, we measured a value of 7.41 ± 0.07‰ (mean ± 1 SE) for five analyses. Overall, external precision on laboratory standards analyzed during the course of this experiment was better than ±0.15‰ for both $\delta^{15}N$ and $\delta^{13}C$.

Isotope analyses for Sr in fish vertebrae and otoliths and stream water from each site have been published (Kennedy et al. 1997, 2000). Briefly, Sr isotopic compositions were measured using either the VG-Sector or Finnegan MAT 262 thermal ionization mass spectrometers at Dartmouth College. Reported uncertainties are two times the standard error (2 × SE) calculated from the measurement of 100–300 ratios on each individual sample. The $^{87}Sr/^{86}Sr$ ratios were normalized to $^{88}Sr/^{86}Sr = 0.1194$. Replicate analysis of a National Institute of Standards and Technology standard reference material (SRM-987), using the Finnegan MAT 262 and the VG-Sector, completed during the course of this study yielded mean $^{87}Sr/^{86}Sr$ ratios of 0.710262 (standard deviation (SD) = 0.000013, $n = 32$) and 0.710215 (SD = 0.000062, $n = 12$), respectively.

Results

Uptake and stability of N, C, and Sr isotopes

Incorporation of stream-specific $\delta^{15}N$ and $\delta^{13}C$ values into fish tissue occurred within the first 5 weeks after stocking (Table 1). The isotopic signature that developed within each site was relatively constant between 40 and 107 days after stocking (Fig. 2). Seasonal variability in the $\delta^{13}C$ signature was greater than that in $\delta^{15}N$, especially within the first 2 months. After 60 days the signal was more constant and the signatures of fish at each site remained distinct despite some within-site variation. Reclassification rates for late-season individuals did not change significantly regardless of whether the discriminant functions were constructed using N and C only from late-season individuals (23 of 37 or 62.2% correct) or from all individuals throughout the growing sea-

Table 1. Stable-isotope values in salmon (*Salmo salar*) from the hatchery and collected from the field sites between 40 and 100+ days after stocking.

Site	Fish age (days)	$\delta^{15}N$	$\delta^{13}C$	*n*
Hatchery	0	14.48 (0.79)	–19.84 (0.49)	7
Flood	40	7.92 (0.20)	–21.16 (0.19)	3
	104	9.23 (0.22)	–22.46 (0.44)	9
Marlboro	40	8.71 (0.41)	–20.04 (0.57)	5
	66	8.79 (0.58)	–19.16 (1.22)	6
	93	9.24 (0.20)	–18.88 (0.78)	8
Utley	45	8.72 (0.33)	–23.47 (1.21)	7
	57	8.02 (0.18)	–23.47 (1.04)	10
	104	8.85 (0.30)	–24.89 (0.21)	2
Bethel Gilead	44	8.47 (0.68)	–21.79 (1.18)	9
	66	8.89 (0.45)	–22.21 (0.87)	9
	107	8.84 (0.58)	–22.78 (0.39)	8
Hancock	47	7.75 (0.51)	–21.56 (1.21)	9
	61	8.26 (0.51)	–21.78 (0.73)	9
	79	8.73 (0.61)	–23.74 (1.11)	10
Tweed	49	8.20 (0.49)	–25.26 (1.68)	[illegible]
	75	7.35 (0.12)	–22.83 (0.68)	5
	100	8.28 (0.58)	–23.54 (2.36)	9

Note: Values are given as the mean with the standard deviation in parentheses; *n* is the sample size.

son (20 of 37 or 54.1% correct). In comparison, when all 118 fish from these five sites were reclassified to site based upon discriminant functions, group membership was correctly predicted for 54.2% (64 of 118) of the cases. Kennedy et al. (2000) studied incorporation of stream-specific Sr isotope ratios into fish tissue and found that $^{87}Sr/^{86}Sr$ ratios in the vertebrae and otoliths of juvenile salmon in 29 stream sites in the West and White rivers also differed from the hatchery signal within the first 3 months after stocking and remained stable within a site over seasons and years.

Relationship between $\delta^{15}N$ and $\delta^{13}C$ values in salmon muscle tissue and scales

There was a strong positive correlation between the isotopic signatures in fish scales and muscle tissue (Fig. 3) for both $\delta^{15}N$ and $\delta^{13}C$ values. The dotted 1:1 line in the figures depicts complete equilibration between scales and muscle tissue. The deviation from the 1:1 line only observed for $\delta^{13}C$ (Fig. 3*b*) in muscle tissue and scales indicates that some of the $\delta^{13}C$ signal (but not the $\delta^{15}N$ signal) from the hatchery ($\delta^{13}C = -19.84 \pm 0.49$) was retained in the scales of 4-month-old fish. We showed previously that the $^{87}Sr/^{86}Sr$ signals in otoliths, scales, and backbones of juvenile Atlantic salmon were statistically indistinguishable (Kennedy et al. 2000).

Distinguishing among salmon populations

The different isotopic systems individually distinguished between juvenile salmon from different rearing locations to different extents. We determined this by testing to see how many clusters of distinct isotopic signatures could be identified among fish from the 11 sites for which we had both $\delta^{13}C$ and $\delta^{15}N$ values. Salmon from 3 of the 11 sites carried significantly different $\delta^{15}N$ signals than salmon from the other sites (Table 2; Fig. 4). These differences allowed us to discriminate among four separate groups (or 33%) of salmon across the study sites (Table 2). Differences in the $\delta^{13}C$ signals in fish distinguished between five separate groups (45%). When $\delta^{13}C$ and $\delta^{15}N$ data were combined, salmon from 7 of the 11 sites could be differentiated, permitting us to separate salmon across the region into eight (74%) isotopically distinct groups. Based upon a discriminant-function analysis, group membership of individuals was correctly predicted in 44.3% (74/167) of the cases in which both N and C were analyzed (Table 2). At the group level, the discriminatory power of $\delta^{15}N$ and $\delta^{13}C$ combined is close to that of Sr (83%), which allowed us to distinguish 8 of 8 tributaries in the White River and 7 of 10 tributaries in the West River (Kennedy et al. 2000) (Table 2). In most cases, N, C, and Sr analyses were not performed on exactly the same fish, therefore we were unable to determine the strength of combining all three isotopic systems in a single discriminant-function analysis.

Discussion

This study contributes to the rapidly growing literature identifying unique and valuable uses of stable isotopes as markers of animal movement and habitat use and food-web characteristics (Hansson et al. 1997; Thorrold et al. 2001; Kennedy et al. 2002). Our results demonstrate the feasibility and potential value of using light-stable isotopes (C and N) as markers for juvenile salmon rearing locations. We conducted these studies with hatchery alevins, which underscores the value of using chemical tracers to assess natal origin in fish that are too small to mark using more conventional methods. We also demonstrated that salmon isotopic compositions can be assessed nonlethally in their scales, which could be extremely important for adapting these techniques for use in endangered populations. Finally, two commonly measured isotopes (C and N) used together differentiated salmon from nearly as many locations as isotope ratios of Sr over the first season of growth. Site-specific differ-

Fig. 2. Incorporation of (*a*) N and (*b*) C isotopic signatures 4 months after stocking in two representative study sites, Bethel Gilead (□) and Marlboro (●). Data exist for four other streams, but these two sites exemplify the general trends across all sites. Fry are stocked in May at weights of less than 0.20 g and weigh approximately 1.0–1.5 g at 40 days of age. By September, parr weigh between 4 and 8 g.

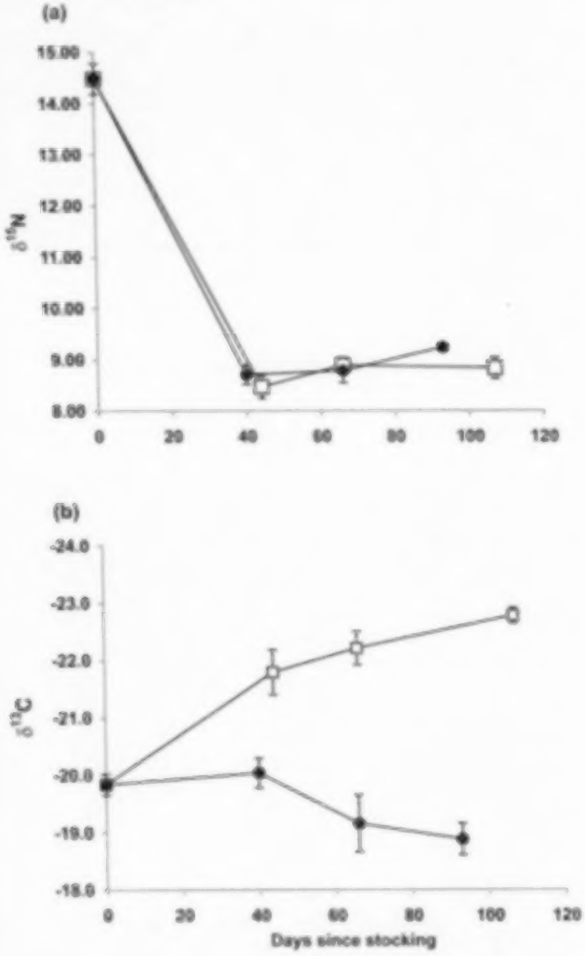
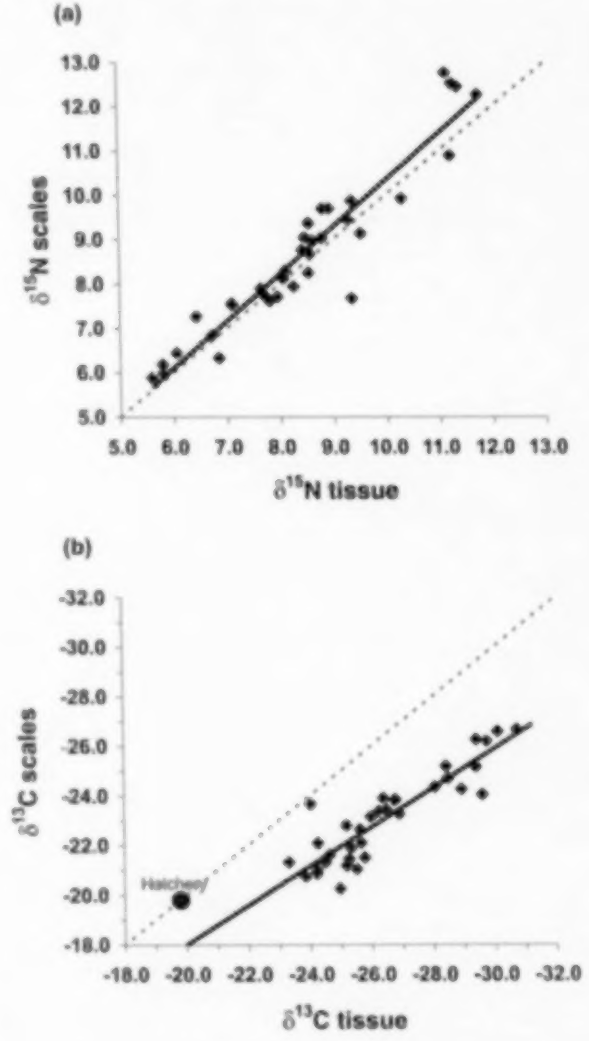


Fig. 3. (*a*) N and (*b*) C isotope ratios in age-0 Atlantic salmon (*Salmo salar*) tissue and scales. Each point represents an individual fish from one of six sites. The broken line represents a 1:1 relationship between $\delta^{15}N$ signatures in tissue and scales. There is a significant positive relationship between the isotopic signatures in muscle tissue and that of entire scales ($P < 0.001$). For N, $y = 1.06x - 0.23$, $r^2 = 0.90$, and for C, $y = 0.79x - 2.29$, $r^2 = 0.80$.



ences in these three isotopic systems that relate to land use, food-web structure, and geology will affect their relative suitability as markers of natal origin.

Uptake and stability

All three elements (N, C, and Sr) are incorporated quickly into the tissue of juvenile salmon. As a result of rapid tissue accumulation and turnover, the N- and C-isotope signatures of fish approach a representative stream value within 5–6 weeks after stocking. Of the different isotopic systems considered, our data indicate that the Sr signal is the most stable in fish over time, followed by N and then C. Differences among these isotopic systems arise from differences in their sources, the extent of biological fractionation, and their allocation and quantification in tissues ranging from highly labile (e.g., muscle) to highly conservative (e.g., otoliths).

The Sr isotope values are likely to be the most stable for several reasons. First, the $^{87}Sr/^{86}Sr$ ratio in stream water arises from bedrock geology (Kennedy et al. 2000). Second, there is no biological fractionation of Sr isotopes and the $^{87}Sr/^{86}Sr$ ratios in fish tissues closely match that of stream water (Kennedy et al. 2000). Third, time-specific Sr signatures are stored in otoliths and are not altered by tissue turn-

Table 2. Discrimination of fish from different field sites based on differences in their stable-isotope signatures.

Site	Reference No.[a]	n[b]	$\delta^{15}N$ group[c]	$\delta^{13}C$ group[c]	$\delta^{15}N$ and $\delta^{13}C$ group[c]	Individual reclassification[d]	$^{87}Sr/^{86}Sr$ group[e]
Marlboro	1	22/19	1	1	1	17 of 19	1
Tweed	2	26/23	1	2	2	2 of 23	2
Utley	3	22/19	1	2	2	0 of 19	3
White River	4	8/8	1	3	3	8 of 8	4
First Branch	5	6/6	2	4	4	6 of 6	5
West Branch	6	20/6	3	2	5	1 of 6	6
Bingo Branch	7	15/6	4	2	6	4 of 6	6
Third Branch	8	5/5	1	4	7	5 of 5	7
Bethel Gilead	9	32/32	1	5	8	7 of 32	7
Flood	10	12/12	1	5	8	9 of 12	8
Hancock	11	31/31	1	5	8	15 of 31	9
Total[f]	11 sites	199/167	4 distinct groups	5 distinct groups	8 distinct groups	74 of 167 (44.3%)	9 distinct groups

[a] Reference numbers refers to sites shown in Figs. 1 and 4.

[b] n denotes the number of fish from each site for both N and C (indicated as N/C). In all cases where N and C values were obtained, the analysis was performed jointly on the same individuals; however, in some cases only N was analyzed

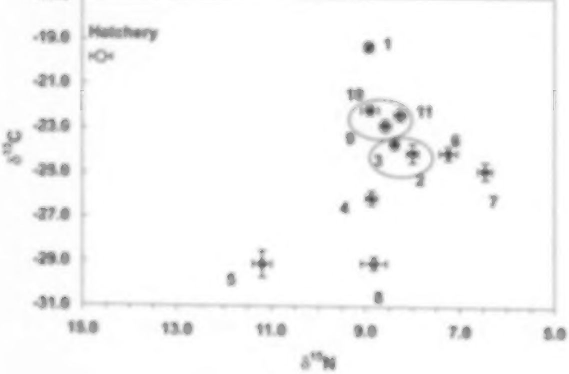
[c] Differences in the isotopic signatures of fish from different sites (pooled within a site over all sampling dates) are denoted by different numbers within each column (based on Fisher's least significant difference multiple comparison as described in the text).

[d] Individual reclassifications are based on the numbers of individual for which group membership was correctly predicted using the results of a linear discriminant function analysis for N and C.

[e] Sr groupings are included for comparison and data are from Kennedy et al. (2000).

[f] The number of sites that could be statistically distinguished by different isotopic signatures.

Fig. 4. Discrimination between the 11 populations of salmon in this study, based on a combination of N- and C-isotope ratios. The open symbol represents the initial hatchery value. When N and C isotopes were used together, discrimination of populations was more than twice that of either method on its own. Two groupings of sites that could not be distinguished on the basis of their coupled isotopic signatures are circled.



over. Recent work shows that time-specific Sr signatures are recorded in otoliths and can be measured with high precision to reconstruct the movements of fish (Kennedy et al. 2002). Constraints on the successful use of this isotope are the spatial heterogeneity of the underlying geology and the relatively high labor intensity of analyzing Sr isotopes.

In contrast to Sr, N undergoes mass-dependent fractionation (~3.5‰) at each trophic level (Deniro and Epstein 1978; Minagawa and Wada 1984; Robinson 2001). Hence, the $\delta^{15}N$ value for an individual at any point in time is based upon the trophic level from which that organism is feeding, which can change within or between seasons (Olson and Young 2003). N-isotope signatures were relatively stable in the fish from our six sites across the entire summer growing season. Despite fairly large increases in biomass, salmon in these streams appear to forage at the same trophic position throughout their first summer, which is consistent with our earlier work on the composition of the diet of juvenile salmon from these sites (Folt and Parrish 1994; Nislow et al. 1999). Hence, the differences in $\delta^{15}N$ values that we measured among sites appear to be more strongly driven by exogenous factors, such as differences in land use, which can create large differences in the $\delta^{15}N$ signal of stream water (Harrington et al. 1998), than by site-specific trophic differences or fish movements.

The C-isotope signature of fish changed the most over the course of the growing season. Based upon the quickness with which the N-isotope signal stabilizes, we believe the seasonal changes in C isotopes to reflect changes in the environment rather than a more gradual attainment of an equilibrial stream signature. These changes likely reflect either a change in the relative contribution of autochthonous and allochthonous C sources to the stream food web in particular locations (Rounick et al. 1982; France 1995*a*) or a seasonal change in the dissolved inorganic C profiles over algal substrates (Finlay et al. 1999). Neither stomach-content analysis (Folt and Parrish 1994) nor $\delta^{15}N$ values (this study) support a trophic shift during this period. A number of studies have used differences in the natural abundance of $\delta^{13}C$ to determine the source of C to the food web, and have shown that as this source varies, the signal in the consumers will also vary (Hamilton et al. 1992; Peterson et al. 1993; Doucett et al. 1996). The rate at which the tissue isotopic signature responds to changes in environmental input depends on the turnover rate in the tissue. Turnover and isotopic equilibration can take from days to weeks in muscle tissues (Hobson and Clark 1993; Herzka and Holt 2000). In this study, we investigated δ^{13}C signatures in the muscle tissue of rapidly growing salmon. Tissue turnover coupled with changes in the primary C source in some streams over time could easily explain the seasonal changes that we measured in δ^{13}C. However, as in the case of δ^{15}N, among-site differences in δ^{13}C were generally greater than the differences occurring within a site over the growing season, which is the period in which we are primarily interested. Consequently, δ^{13}C and δ^{15}N signatures provided short-term markers for salmon habitats over the summer growing season and would provide site-specific otolith signatures where lethal sampling was possible.

Nonlethal sampling

By investigating isotope ratios in the scales of the juvenile salmon, we tested the feasibility of nonlethal sampling of field-caught animals. We found a reliable correspondence between the δ^{15}N and δ^{13}C signature in scales and that in muscle tissue, which suggests that scales can provide a nonlethal marker for isotopic signatures in fish over short time scales. In the case of N, the slope of the line falls very close to the 1:1 line, suggesting that scale turnover is complete and in equilibrium with the relatively rapid exchange in muscle tissue. For C, an offset between the tissue and scale signatures is consistent with some residual hatchery signature being stored in the scales even after tissues have exchanged with the environment.

Our work with Sr isotopes in scales suggests that they also closely track the isotopic signature of their surrounding environment (Kennedy et al. 2000). Similarly, Wells et al. (2000*a*, 2000*b*) found that the trace elements (Sr, Cd, and Ba) in scales were incorporated in concentrations that were in relatively constant proportion to concentrations in the ambient water. Both of these studies analyzed only bulk scale material. Because the C signature from the hatchery appears to be retained in the scales of 3-month-old fish, scale annuli potentially serve as a time-specific repository for past signatures in a similar fashion to otoliths (Campana and Thorrold 2001; Kennedy et al. 2002). Thus, for applications that require the tracking of individual fish over long time periods (e.g., tracing the natal history in a migrating adult), δ^{13}C signatures or trace elements found in measurable concentrations in the annuli of scales may serve as markers. Future studies must address the time-specific storage of these signatures in scales to determine their relative success at providing reliable geochemical markers over the lifetime of fish.

Stock discrimination

All three isotopic systems successfully differentiated salmon, but they varied considerably in their discriminatory power. The δ^{15}N values of resident salmon differentiated approximately 33% of the randomly chosen salmon rearing streams. N isotopes are likely to be most valuable for differentiating fish that were reared in landscapes with varying degrees of agricultural impacts (as described in Harrington et al. 1998; McKinney et al. 2001). The sites used in this study varied only slightly with respect to land use, and no site was greatly impacted by agriculture (e.g., >15% by area). Therefore, our estimate of site discrimination is likely to be conservative when compared with its application in a heterogeneous landscape with a broad range of land uses represented.

In comparison, δ^{13}C differentiated 45% of the sites. These site-to-site differences likely arise from differences in the C sources of the food web (i.e., benthic algae versus allochthonous plant material), the concentration of dissolved inorganic C available to algae, the prevalence of C_3 and C_4 plants in the watersheds, and the geologic contribution to the isotopic composition. When applied randomly across the landscape (as in this study), the discriminatory power of C isotopes was good. Like N isotopes, C isotopes could also be selectively used to distinguish between fish from regions with known differences in terrestrial to autochthonous inputs. However, unlike N isotopes, C isotopes have been shown to vary over relatively small spatial scales (e.g., 10 to 100s of metres) (Finlay et al. 1999) and could be used to distinguish fish movements over different spatial scales (Folt et al. 1998).

Our overall success in discriminating between fish from different sites was greatest using Sr isotopes. This was true in part because the geology of this region is highly variable and produces a mosaic of ^{87}Sr/^{86}Sr ratios across the landscape (Kennedy et al. 2000). In situations where this is likely to be true, ^{87}Sr/^{86}Sr ratios are a powerful discriminatory tool. However, in combination, δ^{15}N and δ^{13}C also differentiated 73% of the sites, which approached the success of the ^{87}Sr/^{86}Sr ratio alone (83%). This result is particularly important because discrimination of fish stocks may be an important issue where homogeneous bedrock precludes the use of Sr isotopes. Additionally, considerably more laboratories are equipped to measure δ^{15}N and δ^{13}C than ^{87}Sr/^{86}Sr. Hence, isotopic signatures of N and C may be particularly effective markers for juvenile salmon rearing locations.

Although more work is needed to establish the extent to which N and C signatures are preserved over long periods of time, our results suggest that these isotopic systems can be very useful in tracking individuals within at least one season of growth over multiple spatial scales. Examples of promising short-term applications include (*i*) following the dispersal of individuals both between sites and within seasons and (*ii*) identifying the tributary origins of outmigrating salmon smolts or mixed populations of other fish species. Adding other isotopes (such as O or H) to develop a multiple-isotope signature will augment our ability to identify rearing or natal origins of dispersing animals without the cost and biological damage associated with tagging or other invasive measures.

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Effects of riparian canopy opening and salmon carcass addition on the abundance and growth of resident salmonids

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Abstract: We studied the concurrent effects of riparian canopy opening and salmon carcass addition on salmonid biomass, density and growth rates in small streams over 2 years. In each of six streams in the Smith and Klamath River basins in northern California, red alder (*Alnus rubra*) and other hardwoods were removed along both banks of a 100-m reach to increase incident radiation. A second 100-m reach, with an intact canopy, was established in each stream as a light control. Salmon carcasses were added each year to both cut and uncut riparian sections in three of the six streams. Differences in total density and biomass of cutthroat trout (*Oncorhynchus clarki*) and rainbow trout (*Oncorhynchus mykiss*) from pretreatment levels responded positively to canopy removal but were not detectably affected by carcass addition. Differences in specific growth rates of the fish between open and closed canopy reaches were greater in sites without carcasses than in sites with carcasses. In light-limited settings where temperature gains associated with canopy opening are not problematic for aquatic resources, gains in salmonid production might be achieved by selective trimming of riparian hardwoods.

Résumé : Nous avons examiné sur une période de deux ans les effets concurrents de l'ouverture de la canopée du rivage et de l'addition de carcasses de saumons sur la biomasse, la densité et les taux de croissance des salmonidés dans de petits cours d'eau. Les aulnes rouges (*Alnus rubra*) et d'autres essences de bois francs ont été retirés sur les deux rives d'une section de 100 m dans six cours d'eau des bassins versants des rivières Smith et Klamath dans le nord de la Californie afin d'accroître la radiation incidente. Une autre section de 100 m à canopée intacte dans chaque cours d'eau a servi de témoin en ce qui a trait à la lumière. Des carcasses de saumons ont été ajoutées à chaque année dans des sections à rives déblayées et intactes dans trois des six cours d'eau. Il y a une augmentation de la densité totale (par comparaison aux valeurs initiales) de la truite fardée (*Oncorhynchus clarki*) et de la truite arc-en-ciel (*Oncorhynchus mykiss*) consécutive au retrait de la canopée, mais il n'y a pas d'effet décelable de l'addition de carcasses. Les taux de croissance spécifiques des poissons dans les sites à canopée ouverte et fermée diffèrent plus dans les sites sans addition de carcasses que dans ceux où il y a eu addition. Dans les situations où la lumière est un facteur limitant, une coupe sélective des bois francs sur les rives peut augmenter la production de salmonidés à la condition que l'accroissement de la température associée à l'ouverture de la canopée ne soit pas néfaste aux ressources aquatiques.

[Traduit par la Rédaction]

Introduction

Nutrient limitation may underlie the finding that, even in pristine settings, salmonid production in coastal streams of the Pacific Northwest often lags behind that achieved in other salmonid streams worldwide. When compared across broad spatial scales, salmonid production is correlated with ion and nutrient concentrations (Kwak and Waters 1997), often irrespective of stream physical features (Bisson and Bilby 1998). Coastal streams of the Pacific Northwest are typically oligotrophic and have relatively low acid-neutralizing capacity (Welch et al. 1998). The relationship between salmonid production and nutrient concentration does not appear to be direct but arises instead from the effect of nutrient concentrations on primary production, with cascading effects through the food web. When nutrients are in short supply, autochthonous production may be reduced, resulting in reduced availability of invertebrate prey and slower fish growth.

Nutrient limitation of Pacific Northwest streams may be exacerbated by a declining nutrient supply from carcasses of spawned adult salmon, as many regional salmon stocks have

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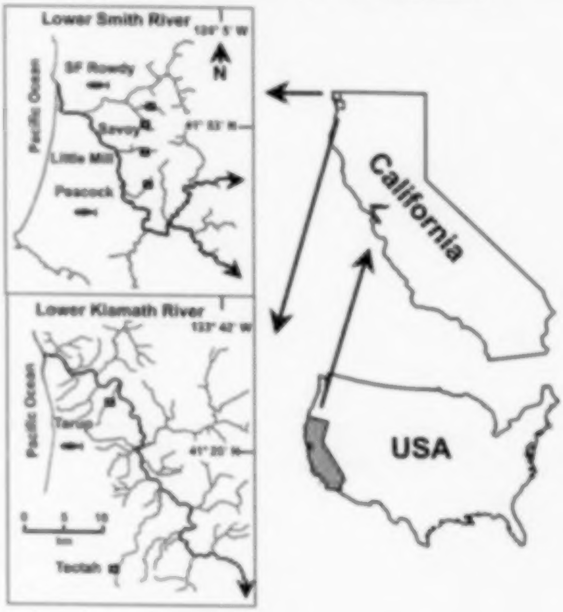
sharply declined (Nehlsen et al. 1991). Gresh et al. (2000) estimated that current salmon returns provide only 6–7% of the historical subsidy of marine-derived nitrogen and phosphorus once delivered to regional rivers. This subsidy results as the anadromous salmon migrate from their marine feeding grounds to spawn and die in their natal streams. Concern that the oligotrophic status of streams is limiting production of juvenile salmonids has prompted the increasing use of carcass introductions as a stream restoration technique (Lackey 2003).

Although several studies have documented that marine-derived nutrients are incorporated into salmonid tissue in freshwater systems (e.g., Kline et al. 1990; Bilby et al. 1996; Chaloner et al. 2002), compelling evidence that salmon carcass introductions affect salmonid population parameters has not yet been established. Only two studies to date have evaluated effects of carcass addition on salmonid densities, biomass, condition factors, or growth. Bilby et al. (1998) compared two Washington streams in which hatchery carcasses were added in late fall to augment carcasses from naturally spawning salmon with paired streams with few spawning salmon. They found that densities and condition factors of age 0+ coho salmon (*Oncorhynchus kisutch*) and age 0+ and 1+ steelhead (*Oncorhynchus mykiss*) increased with carcass addition over the period of salmon spawning and decomposition, but these effects did not persist into spring. Wipfli et al. (2003) found that carcass additions increased growth of age-0 coho salmon in small artificial channels over a 2-month period. Wipfli et al. (2003) also observed that October–May growth of cutthroat trout (*Oncorhynchus clarki*) in a reach of an Alaskan stream where pink salmon (*Oncorhynchus gorbuscha*) carcasses were added in late summer – early fall exceeded the growth of fish in a control reach.

A variety of factors affect the ability of stream salmonids to utilize nutrients provided from salmon carcasses and may dictate the pathways by which carcass-derived nutrients are transferred to salmonids. Carcass-derived nutrients may be transferred to salmonids via (*i*) uptake by autotrophs, which is transferred to fish that feed on primary consumers, (*ii*) substrate sorption of dissolved organic matter and uptake by microfauna, and (*iii*) direct consumption by fish of carcasses, eggs, and fry (Cederholm et al. 1999). Factors affecting pathways of nutrient transfer and the ability of salmonids to benefit from salmon carcasses include stream retentiveness, discharge, consumption by aquatic consumers and terrestrial wildlife, riparian conditions, and ambient water chemistry (Gende et al. 2002). For example, based on laboratory experiments demonstrating uptake of nitrogen by gravel through abiotic processes and comparisons of stable isotope ratios among trophic categories in streams with spawning coho salmon, Bilby et al. (1996) concluded that direct consumption of spawning products by secondary consumers and substrate sorption of dissolved organic matter, but not autotrophic uptake, were important avenues of transfer. However, the streams that they studied were heavily shaded by riparian vegetation, and light limitation of primary production may have influenced their findings. Other studies in fresh water ecosystems have found or suggested that primary production is of primary importance as a pathway to the production of salmonid fishes (e.g., Richey et al. 1975; Mathisen et al. 1988; Kline et al. 1997).

Fig. 1. Location of study sites within the Smith River and Klamath River basins in northern California. The fish symbols identify the three creeks that received salmon carcasses.



Considerable evidence suggests that light limitation of primary production often overrides nutrient limitation in small, forested streams (e.g., Lowe et al. 1986; Rand et al. 1992; Hill et al. 2001). This may be particularly common in the Pacific Northwest, where both coniferous vegetation and an increasing dominance of alder (*Alnus* spp.) (Hu et al. 2001) can provide heavy riparian shade. In coastal settings, summer fog may also reduce light reaching streams.

Where light limits algal production, the ability of stream systems to respond to carcass enrichment may be affected and transfer pathways to salmonids restricted. Autotrophic pathways are particularly important in sustaining salmonid growth during spring and summer (Bilby and Bisson 1992) and are at the basis of the finding that logged streams often support higher salmonid production than their forested counterparts (e.g., Murphy and Hall 1981; Wilzbach et al. 1986). Restoration techniques using carcass introductions to increase growth of juvenile salmonids may fail to fully achieve desired results if factors limiting primary production are not also considered. Previous studies of salmonid response to carcass addition have not concurrently examined the effect of decreasing riparian canopy.

We designed this study to evaluate the response of growth and abundance of resident salmonids to simultaneous manipulations of canopy opening and salmon carcass enrichment, with the expectation that canopy opening and carcass enrichment would have additive positive effects on salmonid density and growth.

Table 1. Geographic coordinates, catchment area, and mean bankful width and channel gradient of open and closed canopy reaches of the study sites.

Stream	Geographic coordinates	Catchment area (km^2)	Mean bankful width (m)		Gradient (%)	
			Open	Closed	Open	Closed
Tectah	41°15′47″N, 123°57′52″W	7.9	6	7.2	2.9	1.7
Tarup	41°27′45″N, 123°59′32″W	4.9	7.9	7.2	2.8	1.8
Peacock	41°50′11″N, 124°5′23″W	3.5	3.8	4.4	2.4	4.2
Little Mill	41°52′27″N, 124°6′47″W	3.4	6.5	5.9	7.7	9.5
South Fork Rowdy	41°55′16″N, 124°5′23″W	4.9	7.9	7.8	5.6	5.1
Savoy	41°54′14″N, 124°5′12″W	5	8	8.6	4.7	5.6

Materials and methods

Study sites

Six second- to third-order streams in coastal northern California were selected for study (Fig. 1): four tributaries of the Smith River (Little Mill, Peacock, Savoy, and South Fork Rowdy creeks) and two tributaries of the lower Klamath River (Tarup and Tectah creeks). Study sites on the six streams were located in stands of 30- to 60-year-old conifers (coastal redwood (*Sequoia sempervirens*), Douglas-fir (*Pseudotsuga menziesii*), and Sitka spruce (*Picea sitchensis*)). Channel morphology of the sites was similar and consisted of alternating riffle–pool sequences with some but not abundant accumulations of large woody debris. Sites were similar in catchment area, stream size, and gradient (Table 1), with riparian vegetation dominated by red alder (*Alnus rubra*) and fish assemblages dominated by resident salmonids (coastal cutthroat trout (*Oncorhynchus clarki clarki*) and rainbow trout/steelhead). In October 2001, salmonid biomass averaged 3.3 $g \cdot m^{-2}$ across all of the study sites (range 2.0–5.0 $g \cdot m^{-2}$, while salmonid density averaged 0.4 $fish \cdot m^{-2}$ (range 0.2–0.7 $fish \cdot m^{-2}$). Sites were selected to be above upstream limits of anadromy for Pacific salmon, as we wished to focus on fishes with extended fresh water residency as well as reduce confounding effects from naturally distributed salmon carcasses. However, we observed small numbers of juvenile coho salmon and chinook salmon (*Oncorhynchus tshawytscha*) in Savoy and South Fork Rowdy creeks in May–June 2002 following a winter with high regional escapement and stream discharge. Neither species was observed in any of the reaches during fall samplings. Other vertebrates present included the coastal (Pacific) giant salamander (*Dicamptodon tenebrosus*), tailed frog (*Ascaphus truei*), and adult foothill yellow-legged (*Rana boylei*) and red-legged (*Rana aurora*) frogs.

The climate in the region is characterized as marine west coast with an annual average precipitation of 170–200 cm, approximately 75% of which falls as rain between November and March. Bedrock of both the Klamath and Smith River study sites is of the Franciscan complex (California Division of Mines and Geology 1964), and soils are of the Hugo-Josephine association (United States Soil Conservation Service 1967).

The experiment was conducted from October 2001 to October 2003. In both water years, US Geological Survey gauging stations on the mainstem Smith and Klamath rivers recorded peak discharges with recurrence intervals of approximately 1 year. Based on ≥70 years of record, discharges during March–June 2003 exceeded mean monthly stream flows but were below them during March–June 2002. Discharges in both basins were below mean monthly stream flows during summer months in 2002 and 2003.

Experimental design

In the field experiment, we supplemented stream nutrients with the addition of salmon carcasses and increased incident light through the removal of riparian hardwoods. The experiment followed a split-plot experimental design, with streams as whole plots and two 100-m reaches within each stream as subplots. The two 100-m reaches were separated by 150–200 m of stream channel. To eliminate the possibility that carcass addition would influence "no-carcass" treatments while retaining the ability to randomly assign treatments, we manipulated carcasses at the whole-stream (whole-plot) scale. Thus, we randomly selected three of the six study streams for carcass addition (Tarup, Peacock, and South Fork Rowdy creeks), and each of the two study reaches in these three streams received carcasses. Within each study stream, we randomly assigned the canopy removal treatment to one of the two study reaches. Stream sections were not blocked during this study. One consequence of this experimental design was that the power of the tests of the canopy removal effect and the interaction of canopy and carcass treatments exceeded the power of the test for a carcass effect.

Carcasses of chinook salmon were introduced into both study reaches in the three selected streams in January of 2002 and 2003. Carcasses were obtained from the California Department of Fish and Game Iron Gate Hatchery for placement in the Klamath River sites and from the private Rowdy

Table 2. ANOVA model used for analyzing fish response to canopy and carcass treatments.

Source of variation	df	Error term
Among streams		
Carcass effect	1	Streams (carcass)
Streams (carcass)	4	
Within streams		
Riparian effect	1	Riparian × streams (carcass)
Carcass × riparian	1	Riparian × streams (carcass)
Riparian × streams (carcass)	4	
Date effect	1	Date × streams (carcass)
Carcass × date	1	Date × streams (carcass)
Date × streams (carcass)	4	
Riparian × date	1	Riparian × date × streams (carcass)
Carcass × riparian × date	1	Riparian × date × streams (carcass)
Riparian × date × streams (carcass)	4	

Creek Hatchery and by hand collection for placement in the Smith River tributaries. The carcasses added to the streams were from salmon that had returned from the sea. They had been spawned prior to placement in the stream, but small quantities of eggs remained in the body cavities of some females. Because mean carcass size differed considerably between the two basins (8.6 kg, Smith River; 5.4 kg, Klamath River), we standardized introductions by mass rather than number to approximately 3 $kg \cdot m^{-1}$ (SD = 0.18) or on an areal basis at 0.94 $kg \cdot m^{-2}$ (SD = 0.33). This exceeds the carcass loading used in other studies of salmonid response to carcass addition (0.56 and 0.62 $kg \cdot m^{-2}$ in Bilby et al. (1998) and 0.83 $kg \cdot m^{-2}$ in the natural stream studied by Wipfli et al. (2003)). Natural carcass densities in streams of coastal northern California are typically less than 100 $fish \cdot km^{-1}$ (Bell 2001; J.B. Waldvogel, University of California Cooperative Extension 586 G Street, Crescent City, CA 95531, USA, personal communication). Carcasses were distributed throughout stream sections in areas of slack water and anchored to the streambed with rebar to ensure that they would not be rapidly flushed from the sections. In the first year of the study, we attached radiotransmitters to an additional six unanchored carcasses within each stream to track their movement.

Riparian hardwood removal involved falling alder and other hardwoods from a 20-m-wide band on each side of the channel. Falling was conducted to minimize damage to the few conifers within the riparian zone. Cut trees were left in place but dragged when necessary out of the active channel. Trees were cut in late December 2001, after leaf drop had occurred, so that we would not affect the supply of litter inputs to the streams from autumnal leaf drop. The canopy manipulation was not repeated in 2002, as little riparian regrowth occurred. Canopy removal was effective in increasing incident radiation. Potential available sunlight received by the streams, estimated using a solar pathfinder (Solar Pathfinder, Pleasantville, Tennessee), did not differ among study reaches prior to canopy removal. After canopy removal, photosynthetically active radiation was measured simultaneously in open and closed canopy reaches with a quantum sensor (LI-190S, LI-COR, Lincoln, Nebraska) on selected dates. Photosynthetically active radiation was generally less than 50 $\mu mol \cdot s^{-2} \cdot m^{-2}$ in closed sections and often greater than 500 $\mu mol \cdot s^{-2} \cdot m^{-2}$ in open sections during daylight hours.

Fish sampling

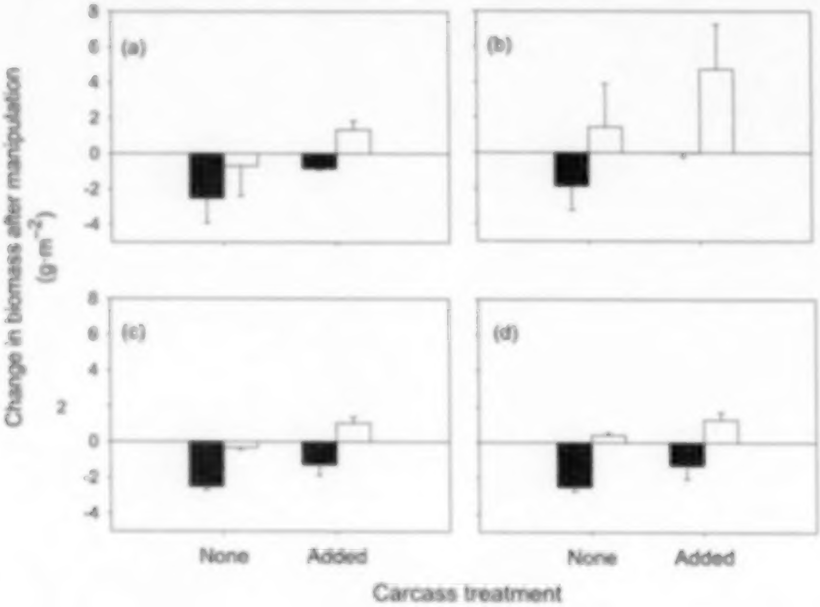
Fish were sampled in all sections by multiple-pass electroshocking in October 2001 (prior to treatment) and in June and October of 2002 and 2003. Each sampling included three passes and, if necessary, additional passes until the number of trout removed was less than 25% of those removed during the previous pass. Captured fish were anesthetized with Alka-Seltzer® tablets before measuring fork length to the nearest millimetre and wet mass to the nearest 0.01 g. Salmonids that could not be identified to species were designated "trout". Individuals greater than or equal to 70 mm fork length were implanted with passive integrated transponder tags on all sampling occasions except in June 2003 and scanned for the presence of tags in June and October of 2002 and 2003.

Other measurements

Stream temperature was monitored to evaluate its response to canopy opening. We deployed submersible temperature data loggers (Stowaway TidbiT) (Onset Computer Corporation, Bourne, Massachusetts) in each stream section prior to manipulation and collected data throughout the experiment. Over a 2- to 3-week period in September 2002, we evaluated the longitudinal extent of temperature effects by deploying additional temperature recorders at the top and bottom of upper stream reaches, 150 m below upper reaches, the bottom of lower reaches, and 130–150 and 350 m below lower reaches.

Grab samples of water from all study reaches were analyzed for concentrations of nitrate (NO_3^-), ammonium (NH_4^+), total nitrogen (TN), soluble reactive phosphorus (PO_4^{3-}), total phosphorus (TP), and silica (SiO_2) in 2002 following carcass addition. Two grab samples were taken at each study reach in late January, mid-March, late April to early May, and late June 2002, corresponding to 2, 6, 15, and 22 weeks following carcass addition. Samples were run using an AutoAnalyzer II (Technicon Industrial Systems/Tarrytown, New York) system following the standard methods (American Public Health Association 1999).

Fig. 2. Change in biomass of all salmonids (coastal cutthroat trout and rainbow trout, age classes combined) in (*a*) June 2002, (*b*) October 2002, (*c*) June 2003, and (*d*) October 2003 from the October 2001 (pretreatment) sample (solid bars, uncut riparian; open bars, cut riparian). Vertical lines above or below bars represent standard error; *n* = 3 stream reaches for each treatment combination on each date.



Data analysis

Salmonid response variables included total and age-specific abundance expressed as biomass and density as well as specific growth rates of post-age-0 fish. Abundance was expressed as the difference in biomass (grams per square metre) or density (fish per square metre) of all salmonids between pretreatment samples (October 2001) and subsequent posttreatment samples (June and October in 2002 and 2003). Specific growth rate (G) of passive integrated transponder tagged individuals was measured as $G = ((\ln W_t - \ln W_0)/t) \times 100$, where W_t is the final mass (grams), W_0 is the initial mass (grams), and t is the growth period (days). Growth was estimated for the overwinter (October 2001 – June 2002) and oversummer (June 2002 – October 2002) periods of 2002 and the overwinter period (October 2002 – June 2003) of 2003. Response variables were analyzed by three-factor ANOVA, with carcass treatment (with and without introduced carcasses), riparian cover (open and closed riparian cover), and date (June 2002, October 2002, June 2003, and October 2003) as factors, with repeated measures on riparian cover and date (Table 2) (Winer et al. 1991). Analyses of specific growth rates included only three levels of date (June 2002, October 2002, and June 2003).

Differences between cut and uncut riparian sections of streams in mean winter (mid-December through mid-March) and summer (mid-June through mid-September) temperature and maximum weekly average temperatures were tested with two-tailed paired t tests on postmanipulation data from the first year of the study. Treatment effects on water chemistry variables (NO_3^-, NH_4^+, TN, PO_4^{3-}, TP, and SiO_2) were analyzed by three-factor ANOVA as described for salmonid response variables. Levels of date for these analyses were late January, mid-March, late April to early May, and late June 2002.

Results

Fate and decomposition of salmon carcasses

A majority (14 of 18) of unanchored, radiotagged salmon carcasses remained within the study reaches and exhibited little displacement from the point of introduction. Ten (56%) of these carcasses were scavenged, and the remainder decomposed within the stream. Only three of 243 anchored carcasses in the first year of the study, however, showed evidence of vertebrate scavenging over a 10-week period of carcass inventory. Both anchored and unanchored salmon carcasses largely decomposed within 1 month after introduction.

Salmonid responses to treatments

The change in total salmonid biomass from pretreatment levels differed between cut and uncut riparian canopies (P = 0.02) and among sampling dates (P = 0.01) but did not detectably differ between carcass-enriched and unfertilized streams (P = 0.32). None of the interaction terms in this analysis achieved statistical significance (all $P > 0.43$). Irrespective of carcass treatment or date, reaches with cut riparian canopies were always associated with a more positive change in biomass compared with uncut reaches (Fig. 2). In cut reaches, biomass increased in all treatment combinations except for streams without carcass additions during the

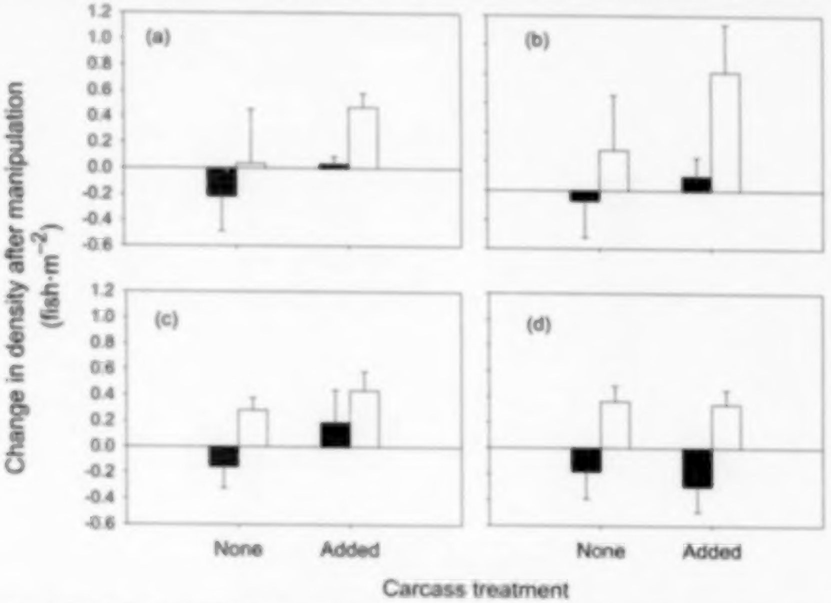


Fig. 3. Change in density of all salmonids (coastal cutthroat trout and rainbow trout, age classes combined) in (*a*) June 2002, (*b*) October 2002, (*c*) June 2003, and (*d*) October 2003 from the October 2001 (pretreatment) sample (solid bars, uncut riparian; open bars, cut riparian). Vertical lines above or below bars represent standard error; $n = 3$ stream reaches for each treatment combination on each date.

overwinter period (June 2002 and June 2003 samples). In uncut reaches, biomass always decreased.

Age-specific analyses of salmonid biomass revealed that neither young-of-the-year ($P = 0.61$) nor post-young-of-the-year (yearling and older) ($P = 0.20$) responded detectably to carcass addition, but these age groups differed in their responses to the other factors. The biomass of young-of-the-year fish was not strongly affected by canopy opening ($P = 0.07$), but biomass differed among dates ($P < 0.01$). Predictably, the biomass of young-of-the-year fish was higher in October than in June in both years. Conversely, biomass of post-young-of-the-year salmonids was higher in reaches where riparian canopies were cut ($P = 0.01$) but did not differ over the four posttreatment sampling dates ($P = 0.11$). Age-specific analyses of salmonid biomass did not yield any significant interaction terms.

As with total salmonid biomass, the change in total salmonid density from pretreatment densities differed between cut and uncut riparian canopies ($P = 0.05$) but not between carcass-enriched and unfertilized streams ($P = 0.53$). The change in total density was not affected by sampling date or interaction terms (all $P > 0.15$). Regardless of carcass treatment or date, reaches with cut riparian canopies supported greater increases in salmonid density than reaches with intact canopies (Fig. 3). The effect of riparian canopy on change in density was greater for yearling and older individuals ($P = 0.01$) than it was for young-of-the-year fish ($P = 0.07$). Carcass treatment, date, and interaction terms did not detectably affect the density of either age group (all $P > 0.08$).

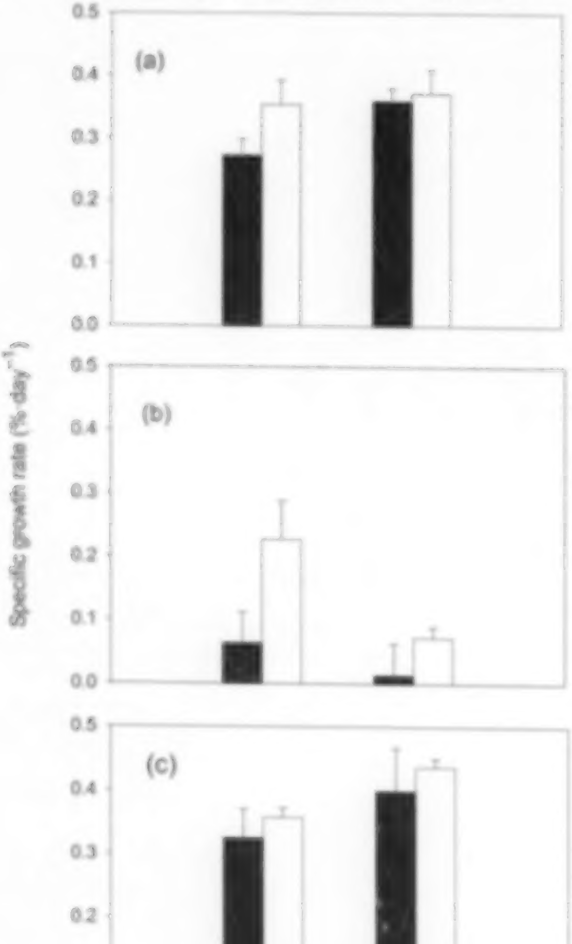
Mean specific growth rates of marked individuals varied among dates ($P < 0.01$) and by treatment combination (carcass × riparian interaction) ($P = 0.02$) (Fig. 4). Fish achieved higher growth in open than in closed canopy reaches (Fig. 4). Differences in growth between open and closed canopy reaches were greater in sites without carcasses (open canopy: mean = 0.31%·day^{-1}, SD = 0.09, $n = 3$ reaches; closed canopy: mean = 0.22 %·day^{-1}, SD = 0.13, $n = 3$ reaches) than in sites with carcasses (open canopy: mean = 0.29%·day^{-1}, SD = 0.17, $n = 3$ reaches; closed canopy: mean = 0.26%·day^{-1}, SD = 0.20, $n = 3$ reaches). Growth was at least threefold greater for individuals recaptured at the end of overwinter periods (June 2002 and June 2003 samples) than it was for individuals recaptured at the end of the oversummer period (October 2002).

Growth results do not appear to have been strongly affected by fish movement, as only one of 572 recaptured individuals was located in a reach different from the reach where it was originally tagged. However, seasonal retention of tagged individuals within study reaches was low, particularly overwinter. Site retention of tagged individuals did not differ between levels of carcass or riparian canopy treatments or their interaction (arcsine square-root transformed data, all $P > 0.29$) but were affected by date ($P < 0.01$). In October 2002, 51% of tagged individuals were recaptured (SD = 15, $n = 12$ reaches). In June 2002 and June 2003, retention was 18% (SD = 8, $n = 12$ reaches) and 12% (SD = 9, $n = 12$ reaches), respectively.

Temperature effects of canopy opening

Cut and uncut riparian reaches did not differ in winter or summer mean temperatures or maximum weekly average temperature (two-tailed paired t tests, df = 5, $P = 0.30$, 0.23, and 0.27, respectively). The difference in maximum weekly average temperature between cut and uncut reaches never exceeded 1 °C (Table 3). Examination of downstream pat-

Fig. 4. Mean specific growth rates of yearling and older PIT-tagged coastal cutthroat trout and rainbow trout recaptured in (*a*) June 2002, (*b*) October 2002, and (*c*) June 2003 (solid bars, uncut riparian; open bars, cut riparian). Vertical lines above bars represent standard error; $n = 3$ for each date and reach-specific mean, which together incorporate 572 separate observations.



terns in temperature during the late summer period in the first year of the study did, however, reveal the persistence of slight temperature increases below reaches with cut riparian canopies. For example, compared with the temperature at the upstream boundary of the cut reach in Tarup Creek, temperature measured 1.5 °C higher at the bottom of the reach (100 m downstream), 1 °C higher 300 m downstream, and 0.5 °C higher 430 m downstream.

Water chemistry response to carcass addition and canopy opening

Experimental manipulations and their interactions had little detectable effect on water chemistry. Carcass addition did not affect concentrations of SiO_2 or species of nitrogen (TN, NO_3^-, and NH_4^+) or phosphorus (TP and PO_4^{3-}) on the dates sampled (all $P > 0.10$). Canopy opening affected only NH_4^+ ($P = 0.01$), with concentrations greater in reaches with open canopies (mean = 0.006 mg·L^{-1}, SD = 0.001, $n = 6$ reaches) than with closed canopies (mean = 0.005 mg·L^{-1}, SD = 0.001, $n = 6$ reaches). Concentrations of SRP and SiO_2 varied with date (both $P < 0.01$) but in an inconsistent fashion. Concentrations of SRP increased steadily from January (mean = 6.18 µg·L^{-1}, SD = 2.53, $n = 12$) to June (mean = 8.6 µg·L^{-1}, SD = 1.90, $n = 12$). SiO_2 concentration was lower in March (mean = 5.85 mg·L^{-1}, SD = 1.99, $n = 12$) than in January (mean = 8.18 mg·L^{-1}, SD = 2.549, $n = 12$), May (mean = 8.33 mg·L^{-1}, SD = 1.97, $n = 12$), or June (mean = 8.87 mg·L^{-1}, SD = 2.27, $n = 12$). Across all dates and treatments, mean concentrations of NO_3^-, TN, and TP were 0.27 mg·L^{-1} (SE = 0.02, $n = 48$), 0.35 mg·L^{-1} (SE = 0.02, $n = 48$), and 9.05 µg·L^{-1} (SE = 0.36, $n = 48$), respectively.

Discussion

In our experiment, the addition of salmon carcasses did not detectably affect total or age-specific salmonid biomass, density, or growth. In contrast, with the exception of the biomass and density of young-of-the-year fish, removal of riparian canopy consistently enhanced salmonid biomass, density, and growth. The strong growth response to canopy opening is particularly salient because it occurred in reaches where fish densities were also greatest. The lack of a strong response to canopy opening by young-of-the-year fish is perhaps not surprising given the clear response of yearling and older fish, which can affect the availability of resources for younger fish. The only significant interaction between carcass and canopy treatments that we detected was that carcass addition reduced the difference in specific growth rates of salmonids between open and closed canopy reaches. Nonetheless, the greatest growth was achieved in open canopy reaches without carcasses.

The finding of a strong canopy effect is consistent with a large number of studies reporting increases in summer biomass and production of headwater salmonid populations after riparian logging (e.g., Hawkins et al. 1983; Johnson et al. 1986; Thedinga et al. 1989). In an experiment in which riparian alder were removed from small Alaskan streams, Keith et al. (1998) observed a reduction in summer abundance of age-0 coho salmon in both open and closed canopy sections, but abundance decreased at a higher rate in closed canopy sections. The general explanation in all of these studies is that canopy opening increased primary production, which appears to be the most important trophic pathway for increasing the availability of aquatic macroinvertebrates preferred by salmonids during spring and summer (Bilby and Bisson 1992). Increased light from canopy opening may also increase foraging efficiency of the fish (Wilzbach et al. 1986).

Table 3. Mean winter and summer temperatures and maximum weekly average temperature in the stream reaches.

			Temperature (°C)		
Stream	Reach	Canopy	Winter mean	Summer mean	Maximum weekly average
Little Mill	Upper	Uncut	8.6	11.9	12.7
	Lower	Cut	8.8	12.2	13.2
Peacock	Upper	Uncut	8.9	12.4	13.3
	Lower	Cut	9	12.8	13.7
Savoy	Upper	Uncut	8.4	12.4	12.9
	Lower	Cut	8.4	12.8	13.5
SF Rowdy	Upper	Cut	8.6	13	13.8
	Lower	Uncut	8.5	13.1	13.8
Tarup	Upper	Cut	9.2	11.7	12.4
	Lower	Uncut	9.3	12.1	13.1
Tectah	Upper	Uncut	7.7	12.1	13.9
	Lower	Cut	7.7	12.9	14.9

Large variation among streams within carcass treatments influenced our ability to detect a carcass effect in this study, and a retrospective power analysis indicated that the ability of our experimental design to detect a significant carcass effect was low. For example, for overall density of fish, the experiment had approximately a 20% probability of rejecting a false null hypothesis of no difference among carcass treatments, assuming one wished to detect a difference of 0.5 fish·m^{-2} between treatments. That increases in mean age-specific and total salmonid biomass and density were always greatest in open stream reaches that also received salmon carcasses suggests that a larger sample size or different experimental design might have detected a significant response of salmonid abundance to carcass addition and that salmonid abundance may respond additively to canopy opening and carcass addition. Our future project plans include an amended design in which carcasses will be added to the lower ends of all six streams to further resolve the extent of any carcass effect. However, the failure of any effect of substantial carcass addition to overcome among-stream variation in salmonid abundance indicates that it may not be a powerful management tool in our study region.

Another potential limitation of this study concerns the spatial scale of investigation. The low overwinter retention of tagged fish within reach boundaries raises the possibility that extensive seasonal movement may have affected the results. This raises questions about the appropriate scale for addressing questions about the effects of carcass and riparian manipulations. Chaloner et al. (2004) emphasized the need to verify previous studies of carcass effects, all of which have been conducted at the reach scale, with studies at broader spatial scales. Because the ability of salmonids to respond to carcass enhancement depends on interactions between physicochemical conditions and biological communities that may be site specific, they believe that variability in salmonid responses to carcass enhancement may extend throughout the Pacific Northwest.

Among the differences that likely contribute to the potential for salmonids to respond to carcass addition are the specific loading of salmon carcasses coupled with the timing of carcass addition relative to stream discharge. The loadings that we used (0.7–1.5 kg·m^{-2}) approached those (1.9 kg·m^{-2}) at which Wipfli et al. (2003) found that increased salmonid growth from carcass additions began to diminish. However, we added carcasses during the wet season in northern California to correspond to the timing of spawning by anadromous salmonids in the study streams. During this time period, flows are generally high and fluctuating, and nutrients provided by carcasses may be flushed from the system before they can be sequestered. This may explain our inability to detect a response in nutrient concentrations to carcass treatments and the failure of Ambrose et al. (2004) to detect a periphyton response in the same stream reaches. A similar carcass loading would likely have a greater effect during low flow. The timing and extent of carcass deposition in the streams that we studied contrast sharply with streams that receive runs of mass-spawning salmon at the end of the dry season such as those represented in experiments by Wipfli et al. (2003) and may contribute to the differences in results between their study and ours. This suggestion corresponds to the recent observation by Bilby et al. (2003) that a nutrient subsidy from anadromous salmon was detectable in riparian vegetation along a Washington stream where dense aggregations of chum salmon (*Oncorhynchus keta*) spawn but not in a nearby stream where coho salmon spawn at lower densities.

Results of this study have important management implications in addition to suggesting avenues of future research. For example, gains in salmonid production might be achieved by selective trimming of riparian alder or other hardwoods if other habitat requirements are met. Selective trimming could reduce light limitation of primary production, resulting in increased availability of invertebrates to support salmonid growth. The importance of light in affecting food supply for fish has been generally undervalued by management agencies, and riparian vegetation that provides as much shade as possible is usually ranked as "better" in habitat evaluations than vegetation that provides less shade. This assumption is likely based on temperature increases that often accompany vegetation removal and the overhead cover that it provides fish. However, temperature increases are not problematic until they approach a level at which they become stressful for the organisms one is hoping to maintain. The magnitude of temperature increase from canopy removal varies by site and harvest treatment (Johnson and Jones 2000) and is affected by both external drivers that influence heat and water delivery to a system and internal hydrological characteristics that determine heat flux within the system (Poole and Berman 2001). The effect of a given temperature increase on salmonid production depends on interactions among growth, food consumption, temperature, and other factors (Railsback and Rose 1999); modest temperature increases need not reduce production. The spatial scale and extent of canopy opening are relevant inasmuch as multiple canopy openings within a watershed have the potential to cumulate thermal loadings downstream (Murphy and Meehan 1991). Additional research could establish the amount and spatial pattern of light gain necessary to optimize local food supplies without sacrificing the beneficial functions that riparian vegetation provides stream and terrestrial ecosystems or reducing salmonid production downstream. Carcass introductions, on the other hand, may fail to enhance salmonid production in settings where light limits primary production or when other factors operate that prevent successful use of carcasses by salmonids. The effectiveness of salmon carcass additions in sustaining salmonid production over at least an annual cycle, or on spatial scales larger than the reach level, has yet to be established. However, salmon carcasses and spawning products provide nutrient subsidies to terrestrial ecosystems, including riparian vegetation (Helfield and Naiman 2001; but see Bilby et al. 2003), as well as to aquatic consumers. The value of carcass introductions in enhancing system productivity becomes more evident at a larger, landscape level (Gende et al. 2002, 2004) than it may be at the level of individual populations.

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Evidence for cumulative temperature as an initiating and terminating factor in downstream migratory behavior of Atlantic salmon (*Salmo salar*) smolts

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Abstract: Temperature control of Atlantic salmon (*Salmo salar*) smolt migration was tested using a novel technique allowing nearly continuous monitoring of behavior with complete control over environmental conditions. Parr and presmolts were implanted with passive integrated transponder tags, placed in simulated streams, and monitored for upstream and downstream movements. Beginning 18 April, temperature was increased 1 °C every third day (advanced), fourth day (ambient), and tenth day (delayed). Smolt downstream movements were initially low, peaked in mid-May, and subsequently declined under all conditions. Parr downstream movements were significantly lower than those of smolts in all treatments (0.8 ± 0.5 movement·day^{-1} versus 26.5 ± 4.5 movements·day^{-1}, mean ± SE) and showed no increase. At delayed temperatures, smolts sustained downstream movements through July; those under ambient and advanced conditions ceased activity by mid-June. Initiation and termination of downstream movements occurred at significantly different temperatures but at the same number of degree-days in all treatments. Physiological changes associated with smolting (gill Na^+,K^+-ATPase activity and plasma thyroxine) were coincident with behavioral changes. This is the first evidence of a behavioral component to the smolt window. We found that temperature experience over time is more relevant to initiation and termination of downstream movement than a temperature threshold.

Résumé : Une nouvelle technique qui permet un suivi presque continuel du comportement des saumoneaux de salmons atlantiques (*Salmo salar*) et un contrôle total des conditions environnementales nous a permis d'évaluer le contrôle exercé par la température sur la migration. Nous avons muni des tacons et des pré-saumoneaux d'étiquettes à transpondeur passif intégré, les avons placés dans des cours d'eau artificiels et avons suivi leurs déplacements vers l'amont et vers l'aval. À partir de 18 avril, nous avons augmenté la température de 1 °C à tous les trois (conditions anticipées), quatre (conditions ambiantes) ou dix jours (conditions retardées). Au départ, les déplacements des saumoneaux vers l'aval sont faibles; ils atteignent un sommet à la mi-mai pour diminuer ensuite dans toutes les conditions. Les déplacements des tacons vers l'aval sont significativement moins importants que ceux des saumoneaux dans toutes les conditions (0,8 ± 0,5 déplacement·jour^{-1} par rapport à 26,5 ± 4,5 déplacements·jour^{-1}; moyenne ± erreur type) et ne subissent aucun accroissement. Aux températures retardées, les saumoneaux continuent leurs déplacements vers l'aval jusqu'à la fin de juillet, alors qu'aux conditions ambiantes et anticipées, les déplacements cessent à la mi-juin. Le début et la cessation des déplacements vers l'aval se font à des températures différentes, mais à la même sommation de chaleur en degrés-jours, dans les diverses conditions. Les changements physiologiques associés à la transformation en saumoneau (l'activité des la Na^+,K^+ adénosine triphosphatase et la thyroxine du plasma) coïncident avec la modification des comportements. Il s'agit de la première fois qu'on démontre l'existence d'une composante comportementale à la fenêtre temporelle de la possibilité de transformation en saumoneau. Nous avons trouvé que l'exposition à la température sur une période de temps explique mieux le début et la fin des déplacements vers l'aval qu'un seuil thermique.

[Traduit par la Rédaction]

Introduction

Before spring emigration, juvenile salmon undergo a dramatic change in preparation for life in the ocean: the parr–smolt transformation (reviewed by Hoar 1988). Changes associated with the transformation have been shown or speculated to be influenced by seasonal changes in environmental conditions, especially daylength (photoperiod) and

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temperature, which both increase in the spring.

Although there is substantial evidence that photoperiod and temperature control smolt physiology, we have only correlative and indirect evidence for the environmental factors controlling downstream migratory behavior of smolts. Field studies have been used to relate environmental factors and population-level downstream movements of salmonids. Downstream migration of Atlantic salmon (*Salmo salar*) smolts has been reported to be initiated at a threshold temperature, 10 °C or slightly above (White 1939; Osterdahl 1969; Solomon 1978). There is also evidence that a temperature threshold does not exist for migratory release of smolts (Jonsson and Ruud-Hansen 1985; Hvidsten et al. 1995). Still other evidence indicates an intermediate role of temperature: where temperature did not seem to have a significant effect on the initiation of migration but seemed to control the subsequent decrease in migratory activity of steelhead trout (*Oncorhynchus mykiss*) (Zaugg and Wagner 1973). Wagner (1974) reported that photoperiod appears to act as a synchronizer of the parr–smolt transformation and when photoperiod is out of phase with temperature, the smolt migration period can be extended or shortened (Wagner 1974). Also, short-term changes in temperature and water flow have been correlated with increased downstream migration in the field (Jonsson et al. 1991; Erkinaro et al. 1998; Aarestrup et al. 2002).

Behavioral differences between parr and smolts include changes in swimming activity, aggressiveness, rheotropism (Godin et al. 1974), schooling behavior (Gibson 1983), diurnal swimming patterns (Thorpe et al. 1988), and critical holding velocities (Graham et al. 1996). Laboratory studies of the parr–smolt transformation have concentrated on differences in behavior between parr and smolts in the spring but have seldom measured downstream migration directly and continuously. Further, changes in behavior between parr and smolt have rarely been studied under controlled environmental conditions.

While there has been no direct experimental evidence of the role of seasonal temperature changes as they affect behavior during the parr–smolt transformation, seasonal increases in daylength and temperature are hypothesized to synchronize physiological and behavioral changes necessary for successful migration to the sea (McCormick et al. 1998). When fish reach the appropriate size-related developmental stage, springtime increases in temperature and photoperiod begin to play a role. Once parr have reached approximately 10–13 cm in autumn, they typically undergo transformation the following spring (Elson 1957). There is a great deal of evidence that some of the physiological factors related to smolting, particularly osmoregulatory changes, can be manipulated by altering photoperiod and temperature. Abrupt increases in daylength result in advances in maximum salinity tolerance and gill Na^+,K^+-ATPase activity (Saunders et al. 1989; McCormick et al. 1996*a*). Warm rearing temperatures significantly advance the date of peak gill Na^+,K^+-ATPase activity with an upper temperature limit for successful smolting being species dependent (McCormick et al. 1996*b*). Duston et al. (1991) also indicated that increases in fresh water temperature accelerate the loss of some smolt characters, notably gill Na^+,K^+-ATPase activity.

There is substantial evidence that the environmental control of both physiological and behavioral changes during smolting is mediated by several endocrine systems (Bern 1978). Plasma thyroid hormones are typically elevated during the transformation (Hoar 1988) and have been associated with behavioral changes such as phototaxis (Iwata et al. 1989) and salinity preference (Iwata et al. 1990). There is some evidence, from electrofished and angled Atlantic salmon smolts (McCormick and Björnsson 1994), trap-captured biwa salmon (*Oncorhynchus rhodurus*) fry (Fujioka et al. 1990), and Arctic char (*Salvelinus alpinus*) smolts (Högasen and Prunet 1997), that plasma thyroxine concentration is elevated in migrants compared with nonmigrants. There is also evidence that thyroid hormone levels do not always increase concurrently with downstream migration in Atlantic salmon smolts (Youngson et al. 1989). The direct relationship between circulating thyroid hormone levels and downstream movements of individuals remains unclear.

Due to the ambiguity involved with correlating natural migration with environmental factors and physiological condition, we developed a novel laboratory approach to continuously examine downstream migration under completely controlled environmental conditions. The objectives of this study were to (*i*) continuously quantify daily downstream movements of individuals undergoing the parr–smolt transformation under controlled conditions, (*ii*) manipulate environmental conditions to determine whether a temperature threshold exists for initiation of migratory behavior of smolts, and (*iii*) assess the link among osmoregulatory physiology (gill Na^+,K^+-ATPase activity), endocrine status (plasma thyroid hormone levels), and behavior that occur under varying environmental conditions.

Materials and methods

Fish maintenance

Atlantic salmon parr from the White River National Fish Hatchery in Vermont were transported in February 1997 to the Conte Anadromous Fish Research Center on the Connecticut River in Turners Falls, Massachusetts. Fish were kept in flow-through 1.5-m-diameter tanks at 4–6 °C until the beginning of behavioral experimentation. Photoperiod was maintained at natural daylength for the latitude of the laboratory (42°30′N). Fish were fed pelleted Zeigler Brothers feed ad libitum before and during experimentation.

Between 7 and 10 March 1997, fish were anesthetized with 100 mg MS-222·L^{-1} buffered with sodium bicarbonate (pH = 7.0). Fork length and weight were recorded for 220 Atlantic salmon juveniles. Fish of 8.5- to 11.9-cm fork length were classified as putative parr (n = 52). Fish of greater than 12.0 cm fork length were classified as putative smolts (n = 168). These lengths and classifications were chosen because parr reaching or exceeding 10.0 cm in length at the end of the first growing season (the previous fall) are most likely to become smolts the next spring (Elson 1957). All fish were tagged internally with a 23-mm-long, 3.4-mm-diameter, 0.6-g passive integrated transponder (PIT) tag with a unique eight-digit code (Texas Instruments). A small incision (approximately 4.0 mm) was made on the ventral surface of the fish between the pectoral fins and a tag was placed intraperitoneally. For fish smaller than 10.0 cm, one suture was made in the incision to ensure closure. Preliminary studies revealed that the incision would close and heal without suturing in fish larger than 10.0 cm and fish of less than 8.5 cm fork length were too small to carry these tags. Fish were allowed to recover for 1 month before being placed into experimental conditions.

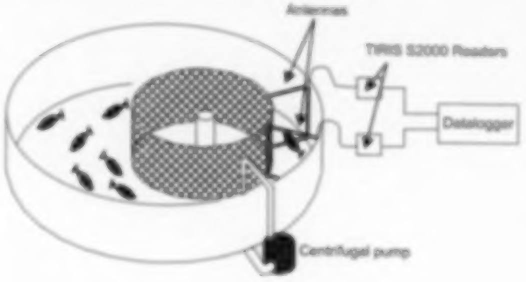
Behavioral experimentation and physiological sampling

On 2 April 1997, length, weight, and PIT tag number were recorded for 75 previously tagged fish. Eighteen putative smolts and seven putative parr were placed in three 1.5-m-diameter circular tanks designed to record upstream and downstream movements of individually marked fish (Fig. 1). Similarly, on 4 April 1997, 120 salmon were split between three 2.4-m-diameter circular tanks (32 putative smolts and eight putative parr in each) designed as above. On both dates, nonlethal gill biopsies were taken for analysis of gill Na^+,K^+-ATPase activity.

Each circular tank had a barrier in the middle and a pump circulated water around the barrier. Water supply to the tanks was flow-through. Water velocity through the narrow portion of the tank was maintained at 50 $cm \cdot s^{-1}$ and ranged from 10 to 40 $cm \cdot s^{-1}$ around the wide portion of the tank depending on distance from the outside wall. Gravel and cobble substrate was placed in the widened portion of the tank and black plastic was attached to the top of the tank to provide shade. Two antennas were built to fit in the narrow portion of each tank. The antennas were 0.3 m apart in the 1.5-m tanks and 0.5 m apart in the 2.4-m tanks. The two antennas were wired to two TIRIS S2000 readers with a different reader energizing each antenna. When in the field of an antenna, a tag would be energized and the reader number, date, time, and tag number were recorded on a computer. The readers were programmed to sequentially switch between pairs of antennas in each of four tanks every 5 min. Tag detections at each antenna were recorded for 2.5 min (12–13 $reads \cdot s^{-1}$) during every 20-min period of the day. Behavioral recordings began on 11 April 1997 and continued until 24 July 1997, except for 20 June when there was a power outage. On 18 April, the maintenance temperature of 4–6 °C was terminated and three temperature regimes were initiated: ambient, advanced, and delayed (Fig. 2). Treatments were achieved by changing the amount of chilled, heated, and ambient Connecticut River water sources into the tank but maintaining constant overall flow rate for all tanks. Based on spring temperature changes over the previous 5 years, the ambient group experienced approximately a 1 °C increase every fourth day until 26 May when the temperature reached 16 °C. The advanced group experienced approximately a 1 °C increase every third day until 10 May when the temperature reached 16 °C; this rate of increase is rare but does occur at this latitude. The delayed group experienced approximately a 1 °C increase every tenth day; this rate of increase simulates a late spring at this latitude or a more typical spring at a northern latitude. Each temperature regime was simulated in two tanks, one 1.5-m and one 2.4-m tank.

On 19 June length, weight, PIT tag number, nonlethal gill biopsies, and nonlethal blood samples were taken from approximately eight smolts in each tank. Gill biopsies were used to determine gill Na^+,K^+-ATPase activity and plasma samples were used to determine thyroxine (T_4) and 3,5,3′-triiodo-L-thyronine (T_3) concentrations. All fish were returned to their original tanks to analyze behavior subsequent to physiological sampling. Physiological sampling of the fish was not conducted before 19 June in an effort to maintain the integrity of the behavioral data being collected.

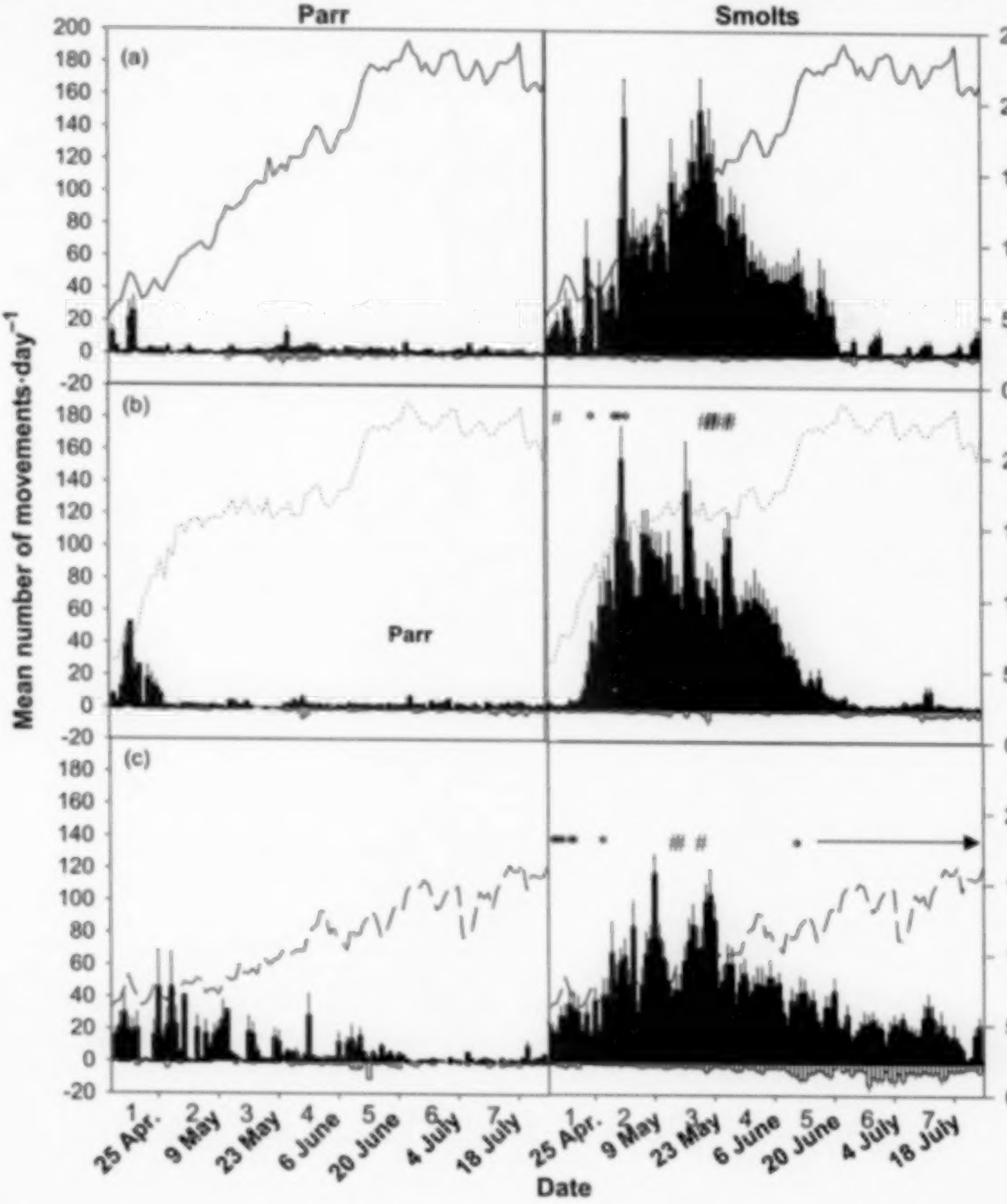
Fig. 1. Schematic representation of the circular stream tank used to monitor movements of individual Atlantic salmon. A barrier to water flow is shown as a cross-hatched structure in the center of the tank. The barrier helped produce unidirectional water motion throughout the tank. Gravel and cobble substrate was on the bottom of the tank in the widened portion. Two antennas, encircling the water column in the narrowed region of the tank, were wired to readers and a datalogging computer.



Behavioral experiments were terminated on 24 July. Length, weight, PIT tag number, gill biopsies, and blood samples were taken from all fish. Sex was determined for all fish. Based on morphological appearance (presence–absence of parr marks, degree of silvering, darkening of fin margins) and gill Na^+,K^+-ATPase activity levels, it was evident that some of the "putative" parr underwent the parr–smolt transformation. Rather than reclassifying these fish as smolts a posteriori, they were removed from the analyses. Similarly, some fish less than 13.0 cm fork length did not demonstrate physiological and morphological smolt characteristics. Therefore, parr and smolts were more conservatively classified as fish between 8.5 and 10.5 cm fork length in April (9.1 ± 0.2 cm, 7.5 ± 1.0 g) and fish greater than 13.0-cm fork length in April (14.5 ± 0.1 cm, 30.2 ± 0.7 g), respectively. These criteria better fit the description of parr–smolt transformation reported by Elson (1957). Only fish meeting these size criteria were used in the data analysis. Twelve fish died in mid-June when temperatures in the advanced and ambient tanks were approaching 24 °C. There was 100% tag retention during the experimental period. Final sample sizes were 15 parr and 26 smolts in the ambient group, 14 parr and 30 smolts in the advanced group, and 12 parr and 26 smolts in the delayed group.

Length, weight, and condition factor were compared using a nested (tank nested within treatment) repeated-measures ANOVA general linear model (GLM) for treatment, developmental state (parr versus smolt), and tank effects. Least square means (LSMeans) tests were performed for pairwise comparisons. Tests were also conducted for smolts alone to test for treatment and tank effects. Specific growth rate from 2 or 4 April to 24 July were compared using a nested ANOVA (GLM) for treatment and tank effects.

Fig. 2. Seasonal daily movements (upstream and downstream) of Atlantic salmon parr and smolts maintained under three temperature regimes: (*a*) ambient, (*b*) advanced, and (*c*) delayed. The *x* axis of each panel indicates the day of the year; each indicated date represents the 2-week period used for statistical analyses (periods 1–7). The *y* axis of each panel is the mean ± SE of the number of movements per individual parr or smolt. Solid bars, positive values, are daily downstream movements and open bars, negative values, are daily upstream movements. The second *y* axis and associated line on each graph indicate the temperatures experienced in the treatment groups. Data are shown separately for parr and smolts from each treatment group. Asterisks and pound signs indicate when values are significantly (ANOVA (GLM) on individual data, $p < 0.05$) greater or less than, respectively, movements in the ambient group. The arrowed line indicates values significantly greater than movements in the ambient group for the period of time indicated by the line.



Behavioral quantification

Movements of parr and smolts were quantified to examine seasonal and daily changes. The frequency distribution of tag detections revealed a cluster of alternating reads below 0.3 s and one above 0.3 s. Those between 0.3 and 3 s were actual movements between antennas, and those below 0.3 s were crosstalk between the antennas and were eliminated from the data set. If the time taken to move between antennas was greater than 3 s, the movement was considered to be around the widened portion of the tank and not direct movement between the antennas in the narrow portion of the tank and removed from the data set. To swim around the inner perimeter of the 1.5-m tanks in 3 s, a 20-cm fish would have to swim at 3.5 body lengths·s^{-1}. Fängstam et al. (1993), with a similar apparatus, reported movements of 2.3 body lengths·s^{-1} during peak migration for 15- to 19-cm Baltic salmon (*S. salar*). Individual daily number of movements (for the 3 h of the day that recordings were made, 7.5 min·h^{-1}; not extrapolated to a full day) was used to examine seasonal patterns of behavior. Individual variation is reported as mean ± SE.

To assess treatment (ambient, advanced, delayed), developmental (parr versus smolt), and tank effects on movements while maintaining individual variation (Ruohonen 1998), a nested (tank within treatment) repeated-measures ANOVA (GLM) was used. Data were not normally distributed and were therefore ranked (lowest to highest using Statistica; StatSoft, Inc.) to conduct the following analyses. The ANOVA design included date as a repeated measure because all individual movements were quantified per day throughout the study. The number of downstream and upstream movements conducted by each individual was summed for each day and used in the repeated-measures analysis. Net movement of individuals was calculated by subtracting the number of upstream movements from the number of downstream movements. Seasonal patterns of downstream movements, particularly for smolts, were not linearly related to time. Therefore, the migratory season was broken into 2-week periods, each of which approached linearity, and separate ANOVAs were run on seven 2-week periods. Nested repeated-measures ANOVAs (GLM) were run for each behavioral period for comparisons of treatment, developmental, and tank effects. When developmental effects were significant, treatment effects were examined for smolts only. Reported significant differences among treatment and developmental groups take into account any tank effects, where differences among treatments or developmental stages are significant above and beyond differences between tanks within treatment group (therefore, possible differences between 1.5- and 2.4-m tank replicates were accounted for in the analyses). LSMeans tests were performed for pairwise comparisons between treatment groups and developmental groups.

To examine the average treatment (temperature) effect on smolt migration, the 7-day running average of the daily number of downstream movements was calculated for smolts in each treatment group. Degree-days were calculated as the cumulative mean daily temperature experienced by the fish since 1 January 1997. To analyze the relationship between degree-days and downstream movement, the transition period from low to elevated downstream activity was defined as the date where smolt downstream movements exceeded the 95th percentile of parr movements (20 downstream movements per day). This was defined as the date that the "behavioral" smolt window opened. For each treatment regime, an endpoint to the window was specified as when smolt downstream activity returned to within the 95th percentile of parr movements. The date that the smolt window opened and closed, the temperature and degree-days experienced on open and close, and the number of days that the "behavioral" smolt window remained open were compared for all three treatment groups. Kruskal–Wallis and Kolomogorov–Smirnov paired tests (when appropriate) were used because some of the data were not normally distributed and did not have homogeneous variances.

Physiological measurements

Approximately two primary gill filaments taken from fish in April, June, and July were placed in individual tubes with 100 µL of ice-cold SEI buffer (250 mmol sucrose·L^{-1}, 10 mmol Na$_2$EDTA·L^{-1}, 50 mmol imidazole·L^{-1}, pH = 7.3) and frozen within 1 h at −80 °C. Gill Na$^+$,K$^+$-ATPase activity was measured using the microassay method of McCormick (1993).

Plasma samples collected in June and July were assayed for T$_4$ and T$_3$. Concentrations were assayed by a direct radioimmunoassay (Dickhoff et al. 1978) as modified by McCormick and Naiman (1984). Intra- and interassay coefficients of variation for these assays were 2.9–10.6% and 12.4–16.2%, respectively.

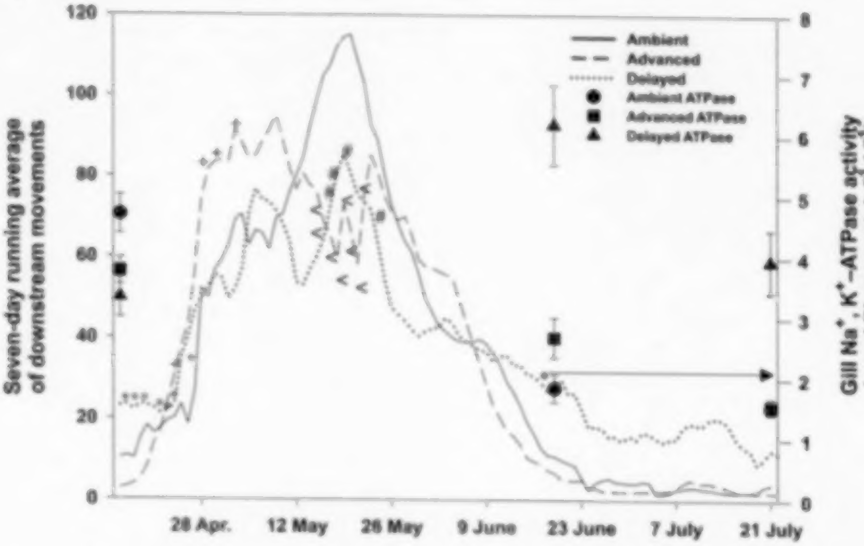
A two-way ANOVA was used to test for treatment and date effects on gill Na$^+$,K$^+$-ATPase activity and plasma thyroid hormone levels. When appropriate, LSMeans post hoc tests were used for multiple comparisons. Data did not meet the assumptions of the ANOVA and were ranked (lowest to highest using Statistica; StatSoft, Inc.) to run the analyses.

Results

Upstream and downstream movements of parr and smolts were continuously quantified from early spring into summer. At the beginning of the experiment (11 April), smolts demonstrated little movement in either the upstream or the downstream direction. Net movement of smolts subsequently became downstream in all groups with an average net movement per day (over the entire experiment) of 26.5 ± 4.5 (n = 82). Net downstream movement of smolts ended in the ambient and advanced treatments by 23 June when levels reached the low levels demonstrated by parr. Parr net movement was also downstream but at significantly lower levels than smolts: 0.82 ± 0.52 (n = 42) (ANOVA, p = 0.0001, df = 1, 116). Parr and smolts differed significantly in the daily number of downstream movements in all behavioral periods, with smolts demonstrating more downstream movements than parr (ANOVA, $p < 0.0005$ for all cases, df = 1, 116).

At ambient temperature, downstream movements increased around 24 April, with a peak occurring during the week of 14 May (Fig. 2*a*). Thereafter, the number of downstream movements declined to reach parr levels by 23 June. The increase in downstream movements far exceeded changes in upstream movements. At advanced temperature, smolts behaved similarly to those in ambient temperature: increased numbers of downstream movements around 16 April with a

Fig. 3. Seven-day running average of the number of downstream movements of Atlantic salmon smolts in the associated temperature treatment groups (mean ± SE). Values for gill Na^+,K^+-ATPase activity are provided for the 3 days when gill biopsies were taken from individuals. Asterisks and pound signs indicate when delayed-group downstream movement values are significantly (ANOCA (GLM) on individual data, $p < 0.05$) greater or less than, respectively, movements in the ambient group. Plus and less than signs indicate when advanced-group downstream movement values are significantly greater or less than, respectively, movements in the ambient group. The arrowed line indicates values in the delayed group significantly greater than movements in the ambient group for the period of time indicated by the line.



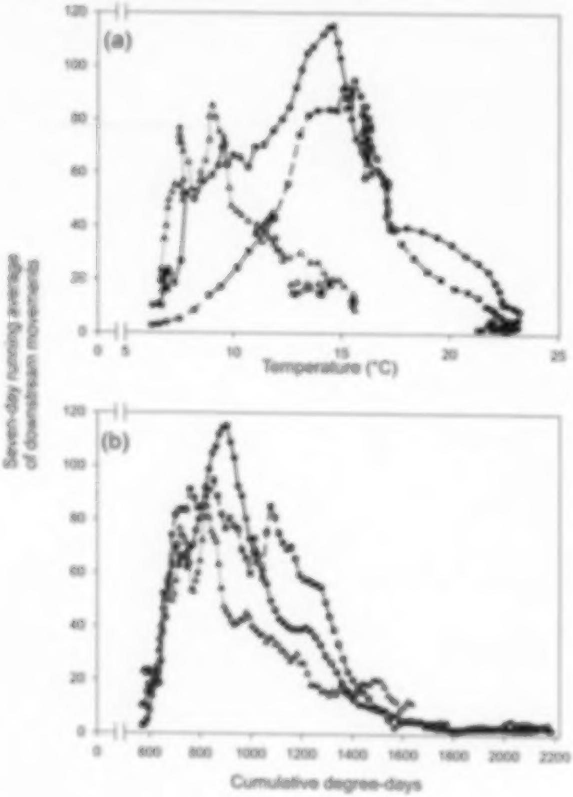
peak during the week of 8 May and a decline to parr levels by 15 June (Fig. 2*b*). At delayed temperature, smolt behavior was very different from that of those in ambient and advanced temperatures: increased numbers of downstream movements around 28 April with a peak during the week of 10 May followed by a slow decline that never decreased to parr levels or smolt levels in the other two temperature groups (Fig. 2*c*).

Temperature treatments resulted in different migratory behavior, as expressed by net movement and downstream movements. The effect of temperature treatment on net movements was significant for all days tested (ANOVA, p = 0.0013, df = 2, n = 116). Temperature effects on net movements were significant during periods 1, 3, and 5–7 (ANOVA, $p < 0.005$, df = 2, n = 116) but not periods 2 (weeks 3 and 4) or 4 (weeks 7 and 8) (ANOVA, $p > 0.05$, df = 2, n = 116). The same effects were seen for downstream movements; temperature effects were significant in all periods except for periods 2 and 4. When the complete distribution of downstream movement data (mean and standard error) was analyzed, there were no significant differences between temperature treatments (ANOVA, p = 0.118 and p = 0.090). However, LSMeans significant differences between the advanced and ambient groups are seen in periods 2 and 4 (Figs. 2 and 3) but do not contribute enough to result in significant differences among all three treatment groups. Significant differences during the last two periods were due to the significantly higher number of downstream movements maintained by smolts in the delayed temperature compared with the low numbers demonstrated by smolts in the ambient and advanced temperatures (LSMeans, $p < 0.05$). Using the 7-day running average of downstream activity, the large difference in the duration of activity in the delayed group was obvious (Fig. 3). Smolts in the delayed group remained above the 95th percentile of parr downstream movement until 3 July, while smolts in the ambient and advanced groups declined to the 95th percentile of parr downstream movement levels by 15 and 10 June, respectively.

The duration of downstream activity was significantly longer under delayed temperature than at other temperatures (ANOVA treatment effect in periods 5–7, p = 0.001, df = 2, n = 116). This sustained period of downstream movement is evident in the differences between parr and smolt behavior at this time. During the last two periods of the experiment, smolts at ambient and advanced temperatures did not differ from parr in number of downstream movements (ANOVA developmental effect, $p > 0.50$). Smolts experiencing delayed temperature had sustained downstream movements above parr levels during both of these periods (ANOVA developmental effect, $p < 0.0001$, df = 1, n = 116).

The absolute temperature at which downstream activity increased differed significantly among the different temperature treatments, indicating that a single temperature does not initiate downstream movement (Fig. 4). Downstream movements increased at 9.1 °C for smolts at ambient temperatures, at 12.5 °C for smolts at advanced temperatures, and at 7.2 °C for smolts at delayed temperatures (Table 1) (Kruskal–Wallis, $p < 0.0001$). However, cumulative degree-days since 1 January was a better predictor of downstream migratory behavior than absolute temperature alone. At 649–700 degree-days, all smolts initiated downstream movements and there were no significant differences among the groups

Fig. 4. Seven-day running average of the number of downstream movements conducted by Atlantic salmon smolts in the associated temperature treatment groups (circles, ambient; squares, advanced; triangles, delayed) shown as a function of (*a*) temperature and (*b*) cumulative degree-days since 1 January.



in the degree-days at downstream initiation (Table 1) (Kruskal–Wallis, $p = 0.09$, df = 2).

Loss of downstream activity at ambient and advanced temperatures occurred during the first week in June, when temperatures reached approximately 16 °C. A decline, but not a complete loss, is also seen in the delayed group just over 1 week later. Cumulative degree-days also seems to be important when determining the termination of migration (Table 1). There was no difference among groups for the degree-days experienced when the behavioral smolt window closed, 1132–1256 (Kruskal–Wallis, $p = 0.3129$, df = 2), but there were differences for the absolute temperature on closure, 11.8–18.8 °C (Kruskal–Wallis, $p < 0.0001$, df = 2).

Based on morphological appearance in July (silvering, loss of parr marks, and dark fin margins), fish larger than 13 cm in April underwent the parr–smolt transformation. Final length, weight, condition factor, and specific growth rates differed significantly between parr (initial length = 10.5 cm) and smolts (initial length = 13 cm) within treatments. Temperature treatment had a substantial effect on growth dynamics associated with smolting. This effect is evident as lower final weight (ANOVA treatment effect, $p = 0.0065$, df = 3, $n = 56$), condition factor (ANOVA treatment effect, $p = 0.002$, df = 3, $n = 56$), and specific growth rates (ANOVA treatment effect, $p = 0.01$, df = 3, $n = 56$) of smolts experiencing a delayed temperature regime (Table 2).

Temperature treatment had a significant effect on gill Na^+,K^+-ATPase activity (Fig. 3), plasma T_3 levels, and plasma T_4 levels (Fig. 5). Significant differences (two-way ANOVA, treatment effect: $p < 0.0001$, df = 2, $n = 167$; date effect: $p < 0.0001$, df = 2, $n = 167$; interaction: $p < 0.001$) were due to significantly higher gill Na^+,K^+-ATPase levels for smolts at delayed temperatures during the last two sampling periods (LSMeans, $p < 0.0001$, $n = 167$). On the individual level, there was a positive correlation between gill Na^+,K^+-ATPase activity and number of downstream movements of smolts recorded on the day before samples were taken in June (Pearson, $r^2 = 0.35$, $p < 0.0001$, $n = 44$) and in July (Pearson, $r^2 = 0.19$, $p = 0.0001$, $n = 69$). Temperature treatment effects were observed for plasma T_4 levels (Fig. 5*a*) (two-way ANOVA, treatment effect: $p < 0.001$, df = 2, 102; date effect: $p < 0.001$, df = 1, 102; interaction: $p < 0.001$). In June, smolts experiencing delayed temperature demonstrated the highest levels of plasma T_4 (LSMeans, $p < 0.005$, df = 2, $n = 44$). In July, smolts at advanced temperature demonstrated higher levels of plasma T_4 than those at ambient temperature (LSMeans, $p < 0.02$, df = 2, $n = 44$). Temperature treatment also affected plasma T_3 levels (Fig. 5*b*) (two-way ANOVA, temperature effect: $p < 0.01$, df = 2, $n = 102$; date effect: $p < 0.001$, df = 1, $n = 102$; no interaction: $p = 0.43$). On 19 June, smolts experiencing ambient temperature had significantly lower levels of plasma T_3 than those at advanced (LSMeans, $p < 0.01$, df = 2, $n = 108$) and delayed (LSMeans, $p < 0.001$, df = 2, $n = 108$) temperatures. Although there were no pairwise differences (among treatments) on 21 July, the same trends are apparent, with the smolts at delayed temperatures having the highest levels of plasma T_3.

Discussion

This study provides the first direct evidence that different temperature regimes, particularly temperature change experienced over time (degree-days), can modify downstream movement of Atlantic salmon smolts. Under identical environmental conditions, smolts expressed significantly more downstream movements than parr. Smolts that experienced an early and rapid increase in temperature (advanced group) expressed an early peak in downstream movements, but the termination of the migratory season was similar to that of those experiencing a normal temperature increase (ambient group). Most notably, the fish experiencing a late and slower increase in temperature (delayed group) in the spring expressed a similarly timed peak in movement but a longer migratory period. Therefore, an early increase in temperature resulted in an early increase in downstream movement of smolts, whereas sustained cold spring temperatures resulted in a sustained migratory period. Degree-days is a better indicator of the initiation and termination of downstream movement than is absolute temperature. This is in opposition to the idea that there is a temperature threshold for initiation of downstream movement.

Few laboratory studies have examined smolt downstream migratory behavior directly, and no studies have examined the

Table 1. Opening and closing dates, temperatures, and calculated degree-days (mean ± SE) for the "behavioral" smolt window of Atlantic salmon smolts maintained under three temperature treatments in simulated stream conditions in 1997.

Behavioral window	Ambient	Advanced	Delayed
Open date	30 April ± 1.9	27 April ± 1.5	25 April ± 1.8
Close date	5 June ± 2.4	1 June ± 2.9	12 June ± 5.1
Temperature on open (°C)	9.1±0.5a	12.5±0.5b	7.2±0.1c
Temperature on close (°C)	18.8±0.7a	18.4±0.5a	11.8±0.5b
Degree-days on open	688±17.7	700±20.5	649±13.2
Degree-days on close	1196±44.7	1256±50.1	1131±59.1
Days open	36±3.5	35±3.5	48±6.0

Note: Different letters indicate statistical differences ($p < 0.05$) among treatment groups.

Table 2. Initial and final size, condition factor, and growth (mean ± SE) of Atlantic salmon smolts maintained under three temperature treatments in simulated stream conditions in 1997.

Growth information	Ambient	Advanced	Delayed
Initial length (cm)	14.8±.2	14.4±.2	14.3±.3
Final length (cm)	20.6±.3	21.2±.3	20.7±.3
Initial weight (g)	31.9±1.6	29.2±1.5	29.3±1.7
Final weight (g)	104.5±4.3ab	115.1±4.0a	97.7±4.7b
Initial condition factor ($100 \times g \cdot cm^{-3}$)	0.968±0.010	0.966±0.010	0.990±0.011
Final condition factor ($100 \times g \cdot cm^{-3}$)	1.18±0.018a	1.18±0.017a	1.10±0.020b
Specific growth ($g \cdot day^{-1}$)	0.0089±0.0005a	0.0109±0.0005b	0.0091±0.0006a

Note: Different letters indicate statistical differences ($p < 0.05$) among treatment groups.

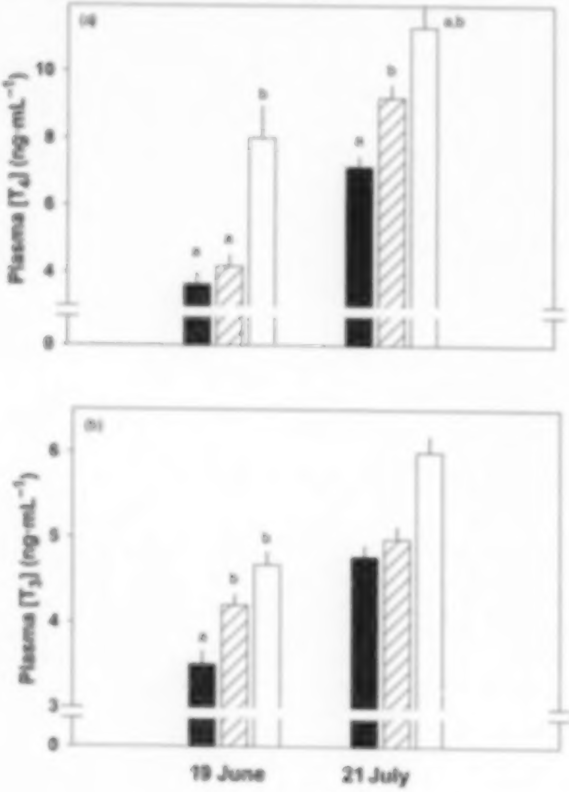
influence of manipulated environmental factors on smolt downstream movements under completely controlled conditions. Wagner (1974) examined downstream movement of steelhead trout. He found that smolts exposed (in the laboratory) to changing temperature were captured at a higher rate in a downstream trap after release than those exposed to constant temperature. Fängstam et al. (1993) examined the effect of early maturation on downstream movement of Baltic salmon using a similar experimental apparatus as used in the current study. Berglund et al. (1994) examined the effect of steroids on downstream migration of smolts of Baltic salmon and found that injection of the androgen 11-ketoandrostenedione resulted in reduced downstream movement of smolts. Lundquist and Eriksson (1985) examined the annual cycle of swimming behavior in previously mature male Baltic salmon with a similar apparatus. They found an increase in downstream movements during spring with a reversal of swimming behavior in late summer under constant photoperiod and temperature, suggesting a circannual rhythm of smolting.

Field studies of smolting salmonids have shown patterns of migration similar to those found in the present study (Jonsson and Ruud-Hansen 1985; Veselov et al. 1998; Antonsson and Gudjonsson 2002). Specifically, Whalen et al. (1999) have shown that peak migration occurs later in the season for tributaries with lower temperature. Also, McCormick et al. (1999) has shown that annual differences in the time to 50% migration of Atlantic salmon smolts from the Connecticut River are related to annual temperature differences. In a field study using release of laboratory-reared fish, Wagner (1974) found that when the temperature cycle was out of phase and behind the photoperiod cycle, the smolt migratory period of steelhead trout was extended (as seen in our delayed treatment), and when temperature was out of phase and ahead, the migratory period was shortened (as seen in our advanced treatment).

Our study indicates that absolute temperature alone is not a regulator of downstream migration because downstream movement is initiated at significantly different temperatures for each of the temperature treatment groups. The temperature effects on migration observed in our study are best explained through the expression of temperature as cumulative degree-days because downstream movement is initiated at the same number of degree-days when fish experience varying temperature regimes. Various environmental factors have been suggested as regulators of migratory release. Some authors have suggested that there is a temperature threshold for the release of migration (White 1939; Fried et al. 1978; Veselov et al. 1998). If field data (particularly Veselov et al. 1998; Antonsson and Gudjonsson 2002) were reinterpreted using degree-days, the link between degree-days and downstream movement may be discovered.

The effect of the interaction between temperature and time, expressed as degree-days, on both the physiology and behavior of smolts may indicate that some feature experienced over time interacts with temperature to regulate smolting. Perhaps the idea that temperature modifies the rate of response to photoperiod for physiological changes (reviewed by Hoar 1988) is also true for behavioral changes associated with smolting. Timing associated with the loss of behavioral smolt characters (i.e., smolt downstream movements returning to parr levels) also had a dependence on degree-days. At the same time, physiological indicators of smolting, particularly gill Na^+,K^+-ATPase activity, returned to low levels late in the season. While reviewing the relationship of smolt physiology and temperature, McCormick et al. (1996*b*) concluded that decreased gill Na^+,K^+-ATPase activity was di-

Fig. 5. Mean and SE of (*a*) plasma T_4 and (*b*) plasma T_3 levels measured on Atlantic salmon smolts from simulated streams maintained at three temperature treatments throughout the migratory season (solid bars, ambient; hatched bars, advanced; open bars, delayed). Statistical differences within each date are indicated by different letters above the bars.



rectly related to degree-days.

Changes in smolt physiology such as gill Na^+,K^+-ATPase activity and salinity tolerance show the same pattern of increase and decline when smolts are maintained in fresh water at high water temperatures (Duston et al. 1991; McCormick et al. 1996*b*) as those demonstrated behaviorally in the present study. From the physiological data, an optimal time frame for migration, controlled by the area's environmental conditions, has been implicated as a physiological smolt window (McCormick et al. 1998). The current study shows that there is not only a physiological limit to the timing of migration but also a limit to behavioral motivation under various environmental conditions that may play a role in the ultimate success of migration to seawater, a behavioral smolt window. Furthermore, as with the opening of the behavioral smolt window, the closure is also likely to be regulated through some interaction of temperature over time. The occurrence and possible relationship between the physiological and behavioral smolt windows have implications for the duration of migration available for seawater entry. For example, if smolts are delayed at dams or kept at hatcheries for extended periods before release, then even if released past dams or into streams, they are not likely to express downstream movements in fresh water at high temperatures or after a certain number of degree-days. Thus, the observed behavioral smolt window indicates that delays in migration may decrease smolt survival based on both physiological and behavioral parameters.

There seems to be a close, although not necessarily causal, relationship between downstream movement and osmoregulatory physiology, as had been previously speculated (Hoar 1988; McCormick et al. 1998). Typically, gill Na^+,K^+-ATPase activity peaks in early to mid-May, depending on annual temperature (McCormick et al. 2000). The patterns of gill Na^+,K^+-ATPase activity in our study followed the same pattern as the behavior. In this study, physiological measurements were not made during the behavioral peak to maintain the integrity of behavioral quantification. While maintaining cooler than normal temperatures in the delayed group, the gill Na^+,K^+-ATPase activity remained high; this relationship was also reported by Sigholt et al. (1998). This is particularly interesting in light of the fact that the number of downstream movements remained high as well, whereas the physiological and behavioral parameters declined in both the ambient and the advanced groups. There was a clear relationship between gill Na^+,K^+-ATPase activity and downstream migratory behavior at the individual and treatment level. Pirhonen and Forsman (1998), examining brown trout and sea trout (*Salmo trutta*) behavior and gill Na^+,K^+-ATPase activity, found a relationship at the population level but not at the individual level. The discrepancy at the individual level may be related to species differences or differences in experimental conditions, as has been seen for brown trout (Nielsen et al. 2004).

Thyroid hormones have been implicated in behavioral changes associated with smolting (Iwata 1995). Although the relationship between changes in T_3 and T_4 levels, temperature, and the continuous expression of downstream movement are first documented here, the relationship between plasma levels and movement as well as the effect of exogenous T_3 and T_4 on behaviors associated with migration have been shown in other salmonids. Exogenous T_4 treatments resulted in decreased aggression of sea-run brown trout, steelhead trout, and masu salmon (*Oncorhynchus masou*), suggesting that T_4 elevation reduces behaviors associated with maintenance of position and thus might also promote downstream migration (Hutchison and Iwata 1998). In the present study, both plasma T_3 and T_4 levels showed similar changes relative to temperature treatment. Furthermore, there was a strong positive correspondence between behavior and plasma thyroid hormones, especially plasma T_4. The present results provide further indirect, correlative evidence for a relationship between thyroid hormones and downstream migratory behavior. Further work will be necessary to provide more direct evidence of the role of thyroid hormones in smolt migration.

Morphological appearance and behavioral characters of parr and smolt seem to correspond, where morphological characters or downstream movement could be used to identify fish as parr or smolt at the beginning of the migratory season. There were distinct behavioral differences between parr and smolts from the end of April until the beginning of

June. Depending on temperature regime experienced, late-season differences in morphology and behavior were not present due to the apparent reversion to parr-level nonmigratory status. In the future, behavioral measures can be used in conjunction with physiological and morphological measures to identify smolt status and potentially to estimate appropriate timing for release of smolts in restoration and enhancement programs.

We have demonstrated that these novel methods can be used to examine questions relative to migration timing as they relate to environmental conditions and critical salmonid life history stages, particularly release and termination of downstream movements of smolts. Previously, these questions have been examined using field data that are correlative and subject to sampling bias (Zaugg and Wagner 1973; Solomon 1978; Muir et al. 1994). Now, questions relating to potential behavioral differences between species, stocks, or smolts of differing physiological condition can be probed using the described laboratory methods.

Physiological parameters associated with smolting have been used to predict survival to seawater entry; however, seawater survival requires a number of specific behavioral expressions, including downstream migration. While indirect field methods have been used to examine the environmental control of behavior, this is the first study to show that the time frame for smolt migration is affected by temperature experience. Our results suggest that in cooler climates and during cooler springs, the smolt migration is likely to last significantly longer than in warmer climates or early springs. This study also provides the first direct demonstration that the expression of downstream movement at the individual level is related to the physiological parameters associated with smolting, connecting the environment, through the neuroendocrine system, to behavioral changes. An important next step to better understand this connection will be manipulation of circulating levels of hormones while monitoring downstream movements. It will also be of interest to examine the genetic component of temperature effects on downstream migration, in particular to examine whether there are river-specific or latitudinal differences in the effect of temperature on smolt migratory behavior.

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Natural chemical markers identify source and date of introduction of an exotic species: lake trout (*Salvelinus namaycush*) in Yellowstone Lake

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Abstract: Exotic species invasions pose a pervasive threat to aquatic ecosystems worldwide, yet fundamental questions about the geographic origin and timing of invasions or introductions are frequently difficult to answer. We used natural chemical markers (Sr:Ca ratios) in otoliths to identify probable source and date of introduction of exotic lake trout (*Salvelinus namaycush*) into Yellowstone Lake, Wyoming, USA. Otolith Sr:Ca ratios were significantly different among lake trout known to have reared in three different Yellowstone National Park lakes (Yellowstone, Heart, and Lewis lakes) and showed little variation along the otolith axis, indicating that lake trout within each lake experienced a similar, and consistent, environmental history. In contrast, suspected transplants showed a large and rapid increase in otolith Sr:Ca ratios indicative of a marked shift to water of differing chemistry. Timing of the abrupt change in Sr:Ca ratios indicated that some lake trout were introduced into Yellowstone Lake during the late 1980s, but more recent transplants also occurred. A discriminant model identified Lewis Lake as the likely source lake for lake trout transplanted into Yellowstone Lake. Our results demonstrate that chemical signatures within otoliths can serve as an important forensic tool for identifying the probable source and date of exotic fish introductions.

Résumé : Bien que l'invasion d'espèces exotiques soit une menace omniprésente dans les écosystèmes aquatiques à l'échelle de la planète, les questions fondamentales concernant l'origine géographique et la date des invasions et des introductions restent souvent difficiles à résoudre. Nous avons utilisé des marqueurs chimiques naturels (rapports Sr:Ca) dans les otolithes pour identifier la source probable et la date d'introduction des touladis (*Salvelinus namaycush*) exotiques du lac Yellowstone, Wyoming, É.-U. Les rapports Sr:Ca des touladis élevés dans trois lacs différents du parc national de Yellowstone (Yellowstone, Heart et Lewis) sont significativement différents et ils affichent peu de variation le long de l'axe de l'otolithe, ce qui indique que les touladis de chacun des lacs y ont vécu une histoire environnementale semblable et uniforme. En revanche, les poissons soupçonnés d'avoir été transplantés montrent une augmentation rapide et importante des rapports Sr:Ca dans leurs otolithes, ce qui indique un passage subit dans une eau de caractéristiques chimiques différentes. La détermination de la date de ces changements abrupts des rapports Sr:Ca indique que certains touladis ont été introduits dans le lac Yellowstone à la fin des années 1980, mais qu'il y a eu aussi des introductions plus récentes. Un modèle discriminant identifie le lac Lewis comme la source probable des touladis introduits dans le lac Yellowstone. Nos résultats montrent que les signatures chimiques des otolithes peuvent être des outils de recherche intéressants pour identifier la source et la date probables d'introduction de poissons exotiques.

[Traduit par la Rédaction]

Introduction

Exotic species pose one of the most pervasive threats to fresh waters worldwide (Hall and Mills 2000; Rahel 2000; Kolar and Lodge 2002). Dramatic changes in species abundance and energy flow have been observed following the establishment of a single new species, even in large lakes (Zaret and Paine 1973; Vander Zanden et al. 1999). Although many exotic species have been intentionally introduced for commercial or recreational purposes, unauthorized transplants and invasions have also contributed substantially to exotic species expansion (McMahon and Bennett 1996; Fuller et al. 1999; Rahel 2000).

When a new exotic species is first discovered, questions about where it originated and when it was transplanted or invaded are frequently difficult to answer with confidence

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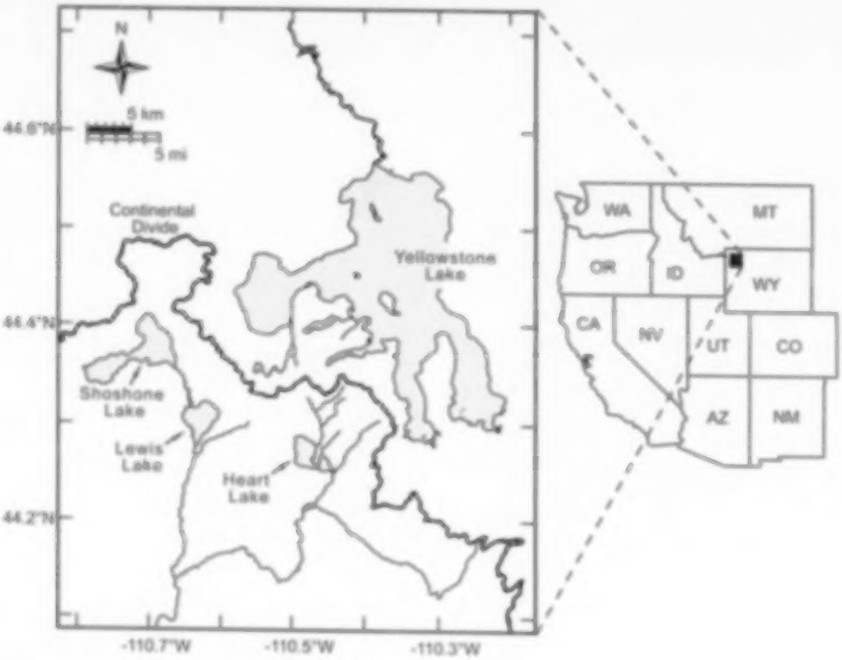
Fig. 1. Map of the major lakes in the study area, Yellowstone National Park.



(Radtke 1995; McMahon and Bennett 1996; Hebert and Cristescu 2002). This uncertainty hinders possible management actions for reducing future occurrences and, in some instances, raises questions as to whether a presumed invader is in fact native or else has resided in the system longer than suspected but at low abundance (Kaeding et al. 1996; Waters et al. 2002). Recent investigations of fresh water zooplankton illustrate the utility of genetic markers as a forensic tool for studying invasion biology (Cristescu et al. 2001; Hebert and Cristescu 2002). In this paper, we demonstrate the use of natural chemical markers in fish otoliths to identify the probable source and date of introduction of an exotic fish species.

Exotic lake trout (*Salvelinus namaycush*) were first discovered in Yellowstone Lake, Yellowstone National Park, Wyoming, USA, in 1994 (Kaeding et al. 1996). This 250 000-ha high-elevation lake near the headwaters of the Yellowstone River drainage is one of the largest relatively intact lake ecosystems in the United States and is the primary remaining habitat for Yellowstone cutthroat trout (*Oncorhynchus clarki bouvieri*) (Gresswell and Varley 1988). The clear, deep, cold waters and abundant prey base of Yellowstone Lake provide prime habitat for piscivorous lake trout, and by 1996, their population was estimated at several thousand, including individuals as large as 91 cm (Ruzycki et al. 2003). Development of an abundant lake trout population was anticipated if left unchecked, with the resultant high predation pressure causing a significant decline in the cutthroat trout population (Varley and Schullery 1995; Ruzycki et al. 2003). Cutthroat trout generally evolved in the absence of competing top predators (Behnke 1992), and similar declines have been documented for several western North American lakes following lake trout introduction (Cordone and Frantz 1966; Marnell 1988; Donald and Alger 1993). Consequently, an aggressive lake trout removal program was initiated in Yellowstone Lake in 1995 to protect a valuable recreational fishery and the integrity of the lake's terrestrial and aquatic foodwebs, which are heavily dependent on cutthroat trout (Varley and Schullery 1995; Koel et al. 2003).

The origin of the lake trout in Yellowstone Lake is unknown. Although lake trout from the Great Lakes were introduced into Yellowstone National Park's Shoshone and Lewis lakes in the late 1800s and later spread to Heart Lake, these lakes are in the Snake River (Pacific) drainage and lack connection to the Yellowstone River (Atlantic) drainage (Fig. 1) (Varley and Schullery 1983). Prior to 1994, no lake trout had been reported in Yellowstone Lake despite extensive population sampling and angler survey records dating back more than 50 years (Gresswell and Varley 1988; Kaeding et al. 1996). Based on the age and size of lake trout when they were first discovered in 1994 (≤5 years and 43 cm), it was estimated that lake trout had reproduced in Yellowstone Lake since at least 1989, but when the original transplant occurred was unknown (Kaeding et al. 1996).

We used chemical analysis of otoliths to estimate where the lake trout originated and when they were transplanted into Yellowstone Lake. Because trace elements of ambient waters are incorporated into otoliths as a fish grows, analysis of natural chemical markers in otoliths can be used to reconstruct environmental history, including timing of movements and stock origins (Campana 1999; Limburg et al. 2001; Thorrold et al. 2001). However, to our knowledge, there have been no previous reports of using the elemental compo-

Table 1. Summary of length, age, and year-class of the known-origin lake trout and suspected transplants collected from three lakes in Yellowstone National Park.

			Total length (mm)		Age (years)		
Group	Lake	*n*	Median	Range	Median	Range	Year-class range
Known-origin	Heart	10	490	327–691	15	8–22	1977–1991
	Lewis	10	503	416–949	14	9–26	1973–1990
	Yellowstone	10	720[a]	700–767	9	8–11	1988–1991
Suspected transplants	Yellowstone	20	805	765–890	18	10–32	1965–1987

[a] Length data were not available for five of the known-origin Yellowstone Lake fish.

sition of otoliths to assess introductions of exotic species. The Sr:Ca ratio has been the most widely used marker in otolith composition studies because (*i*) of the strong correlation between otolith and ambient water ratios, (*ii*) substitution of Sr for Ca in the otolith's crystalline structure, and (*iii*) Sr is more apt to reflect environmental concentrations than other elements owing to a lack of physiological regulation (Campana 1999).

We compared the Sr:Ca ratios in otoliths from suspected transplants with those in (*i*) otoliths of lake trout from more recent year-classes, thought to have been spawned and reared in Yellowstone Lake, and (*ii*) otoliths of lake trout from the two most likely source lakes from within Yellowstone National Park (Lewis and Heart lakes). We hypothesized that lake trout reared in a single lake would have similar otolith Sr:Ca ratios throughout their lives, from the early-growth zone near the nucleus to the outer edge. In contrast, we predicted that lake trout transplanted into Yellowstone Lake would have a significantly different chemical composition between the two zones, reflective of a change in environmental history, and that the Sr:Ca ratio of the early-growth zone could be used to identify the probable source lake of the transplant. We further surmised that among suspected transplants, the timing of the change in Sr:Ca ratio in relation to the age of the fish could provide an estimated date of when transplantation had occurred.

Materials and methods

Otolith collection and preparation

Two groups of otoliths were analyzed for this study. The first group consisted of archived otoliths from lake trout that had been collected from Yellowstone Lake during early stages of the lake trout removal program in 1996 and 1997. It was surmised that the largest fish in these samples were likely some of the original fish transplanted to Yellowstone Lake and smaller sizes were offspring of these suspected transplants. Twenty otoliths, 10 from each year, were randomly selected from among the 164 largest lake trout that constituted the suspected transplant group. These fish were >70 cm total length and comprised the upper 10%–20% of length range of lake trout collected during 1996 and 1997 gillnet sampling (Table 1). The second group consisted of otoliths from lake trout of known origin: suspected offspring of the original founding population in Yellowstone Lake and lake trout of various ages from Heart and Lewis lakes (Table 1). Otoliths from this group were randomly selected from fish gillnetted in 1999 from all three lakes (*n* = 10 for each lake).

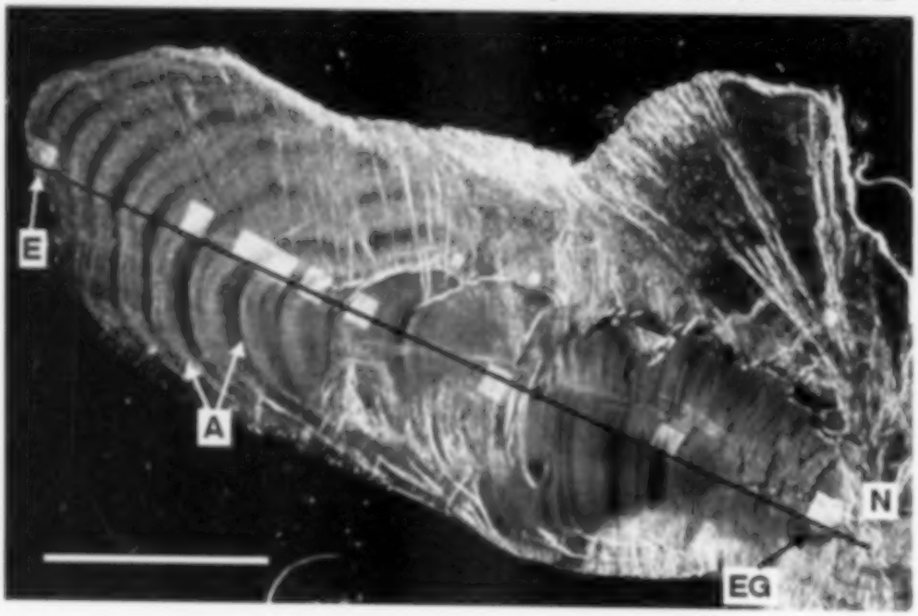
Otoliths were extracted, cleaned, and stored in polyethylene vials soon after collection. One otolith from each fish was sectioned, ground, and polished to expose the nucleus following the techniques of Secor et al. (1992). Prior to chemical analysis, otolith sections were ultrasonically cleaned in a series of baths of Milli-Q water, analytical-grade hexane, and analytical-grade methanol (<1 min each) to remove surface contaminants. Otoliths were then sputter-coated with 15 nm of Au–Pd alloy to reduce the buildup of an electrical charge on the sample during analysis.

Otolith chemistry

Otolith chemical composition was measured with a Phi-Evans (Chanhassen, Minnesota) time-of-flight secondary ion mass spectrometer (ToF-SIMS) (Schueler 1992) at the Image and Chemical Analysis Laboratory, Montana State University, Bozeman, Montana. Positive ion spectra were collected in high-mass resolution mode using a pulsed 15-keV primary Ga^+ ion beam focused to 9 µm × 9 µm. Ion counts for ^{88}Sr and ^{44}Ca were measured from the spectra and reported as Sr:Ca ratios. A laboratory standard consisting of ground and pressed otoliths from hatchery-reared rainbow trout (*Oncorhynchus mykiss*) was analyzed at the start of each session to assess instrument precision, and the ToF-SIMS was recalibrated if the Sr:Ca ratio was >2 SD of the baseline mean of the standard. Within-site sampling variation was estimated by repeat sampling of the same site on 10 randomly selected otoliths. Prior to sampling, a 60 µm × 60 µm area was sputtered for 4 min to remove the alloy coating. Ion counts were measured at the center of the sputtered area.

For each otolith, ion counts were measured at two sites, the early-growth zone near the nucleus and a zone near the outer edge (Fig. 2). For a subset of otoliths from each group (suspected transplants into Yellowstone Lake, *n* = 5; offspring of transplants, *n* = 1; Heart Lake, *n* = 2; Lewis Lake, *n* = 1), additional ion counts were measured at three equidistant points between the early-growth and edge sample sites. If large changes were detected between adjacent sample sites, further sites were sampled to pinpoint the location of temporal changes in Sr:Ca ratio along the otolith axis (Fig. 2). Abrupt changes in Sr:Ca ratio have been correlated with rapid changes in water chemistry (e.g., Limburg et al. 2001). The date of a large change in Sr:Ca ratio, indicative of a movement to new waters, was estimated by comparing the location of the change on the otolith axis with the age of the fish at that time. Annuli were identified using ageing criteria developed for lake trout otoliths by Sharp and Bernard (1988).

Fig. 2. Transverse section of a lake trout (85 cm, 18 years) otolith from a suspected transplant into Yellowstone Lake. Analysis sites for ^{88}Sr and ^{44}Ca ion counts were located in the early-growth zone (EG), near the nucleus (N), and the edge zone (E) in each otolith. Sr:Ca ratio data were obtained from a 9 µm × 9 µm area within the areas cleared of the Au–Pd coating (open squares). Analyses along a transect (solid line) were used to pinpoint the location of any temporal changes in Sr:Ca ratios. A, annuli. Scale bar = 0.5 mm.



Water chemistry

Sr:Ca ratios of water from each of the three study lakes were measured to assess if geochemical differences existed among the lakes and the degree to which these differences were imparted to lake trout otoliths. Surface water samples were collected from Heart and Lewis lakes in 2000 (n = 4 per lake) following standard protocols (American Public Health Association 1998). Water was collected in 1-L polyethylene acid-washed bottles and 100 mL of each sample was immediately filtered through a 0.45-µm-pore membrane filter into an opaque acid-washed polyethylene bottle. Water samples were then preserved with 1 mL of analytical-grade concentrated nitric acid and refrigerated until analyzed. Yellowstone Lake water samples were collected at different depths in the water column in four areas of the lake (Southeast Arm, West Thumb, Mary Bay, and Stevenson Island) in 1997 (n = 30) and 1998 (n = 41) using a hydrobottle clean of trace metals (see Balistrieri et al. 2004). The water samples were filtered and preserved using the same methods described above. Total dissolved Sr and Ca concentrations (milligrams per litre) were measured with a Perkin-Elmer (Wellesley, Massachusetts) Sciex Elan 6000 inductively coupled plasma mass spectrometer (Lamothe et al. 1999) and converted to molar concentrations for calculation of the Sr:Ca ratio.

Statistical analyses

A type III mixed model analysis of variance (ANOVA) and Tukey's multiple comparison tests (SAS Institute Inc. 2000) were used to compare Sr:Ca ratios among known-origin lake trout from Heart, Lewis, and Yellowstone lakes. Lake and otolith zone (early growth and edge) were included as fixed factors and fish, zone × fish interaction, and zone replication as random factors in the ANOVA. Compound symmetry covariance structure (constant variance and covariance) was used to account for possible correlation among multiple measurements from the same otolith (Littell et al. 1998). The Satterthwaite approximation was used to calculate the degrees of freedom for the statistical tests (Littell et al. 1996). For all significance tests, α = 0.05.

Nearest-neighbor discriminant analysis, a nonparametric discrimination procedure (Johnson 1998; SAS Institute Inc. 2000), was used to determine the probable source of lake trout in Yellowstone Lake. Mean otolith Sr:Ca ratios for known-origin fish, weighted by number of sites sampled in each otolith zone, were used to construct the discriminant model (PROC DISCRIM, METHOD = NPAR, k = 9; SAS Institute Inc. 2000). This discrimination procedure assigns each new observation into the group to which the majority of its k nearest neighbors belong (those with the smallest Mahalanobis distances) (Johnson 1998). Model accuracy was evaluated by cross-validation. The Sr:Ca ratios from lake trout otoliths of suspected transplants were then classified using the discriminant model developed for the known-origin data set.

Differences in lake water Sr:Ca ratio among the three study lakes were evaluated using a one-factor ANOVA, and Tukey's multiple comparison test was used to test for pairwise differences. Simple linear regression was used to assess the relationship between otolith and lake water Sr:Ca ratio. Only Sr:Ca data from the otolith edge zones were used to best match otolith composition with lake water composi-

Fig. 3. Relation between mean (±2 SE) lake water Sr:Ca ratios and mean (±2 SE) otolith edge Sr:Ca ratios of lake trout from Heart (▲), Lewis (●), and Yellowstone (■) lakes, Yellowstone National Park. The solid line denotes the fitted linear regression line: $\mathrm{Sr{:}Ca}_{\mathrm{otolith}} = 0.0047 + 0.0088[\mathrm{Sr{:}Ca}]_{\mathrm{water}}$ ($r = 0.997$, $p = 0.037$).

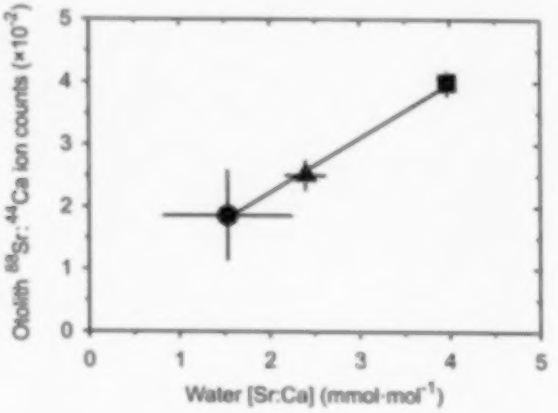


Table 2. Lake water Sr:Ca ratios (mmol·mol^{-1}) from Heart, Lewis, and Yellowstone lakes, Yellowstone National Park.

Lake	Year	n	Mean (SD)
Heart	1999	4	2.40 (0.23)
Lewis	1999	4	1.53 (0.71)
Yellowstone	1997	30	3.92 (0.10)
	1998	41	4.01 (0.14)

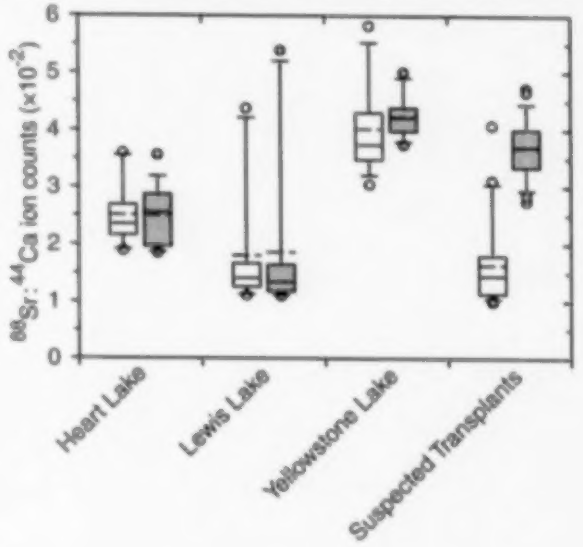
tion at the times of sampling (within 1 year for Heart and Lewis lakes and 2 years for Yellowstone Lake).

Results

Lake water Sr:Ca ratios were significantly different among Heart, Lewis, and Yellowstone lakes (ANOVA, $F = 402.21$, df = 2, 76, $p < 0.001$) with differences in mean values among the lakes ranging from 160% to 270% (Fig. 3; Table 2). Tukey's multiple comparison tests indicated that there were significant differences ($p < 0.05$) for all pairwise comparisons among the lakes. There was a significant linear relation between the otolith Sr:Ca ratios of known origin lake trout and the lake water Sr:Ca ratios ($r = 0.997$, $p = 0.037$) (Fig. 3). Although possible year effects could not be ruled out because water samples from Lewis and Heart lakes were collected in a different year than Yellowstone Lake samples, small differences in lake water Sr:Ca between the 1997 and 1998 water samples in Yellowstone Lake suggest that annual variation in water chemistry was minor compared with the among-lake differences (Table 2).

Otolith Sr:Ca ratios of known-origin lake trout were significantly different among the three lakes ($F = 28.84$, df = 2, 27.9, $p < 0.001$) (Fig. 4). Mean otolith Sr:Ca ratios of Yellowstone Lake lake trout were significantly different from lake trout otolith Sr:Ca ratios from Heart Lake (Tukey's test, $t = -5.10$, df = 28, $p < 0.001$) and Lewis Lake ($t = -7.43$, df = 28, $p < 0.001$). Differences in otolith Sr:Ca ratios between lake trout from Lewis and Heart lakes were not significant at the $\alpha = 0.05$ level ($t = 2.33$, df = 27.8, $p = 0.068$). However, the Sr:Ca ratio for Lewis Lake fish was strongly influenced by one fish with a Sr:Ca ratio much greater (3.7 SD) than that observed in the other nine fish sampled (Fig. 4). When this outlier was removed, the pairwise difference in otolith Sr:Ca ratios between Lewis Lake and Heart Lake lake trout was highly significant ($p < 0.001$).

Fig. 4. Comparison of Sr:Ca ratios of known-origin lake trout otoliths and otoliths of suspected transplants. Two zones were analyzed for each otolith: early-growth (open boxes) and edge zones (shaded boxes) for both known-origin lake trout from Heart, Lewis, and Yellowstone lakes ($n = 10$ per lake) and suspected transplants gillnetted from Yellowstone Lake ($n = 20$). Boxes show the mean Sr:Ca ratios (broken line), median (central solid line), first and third quartiles (box edges), and individual outliers (circles) outside the 10th and 90th percentiles (whiskers).



Otolith Sr:Ca ratios between the early-growth and edge zones among known-origin lake trout from each lake varied little (ANOVA, $F = 1.17$, df = 1, 28.5, $p = 0.289$) (Fig. 4), the average percent difference ranging from 0.1% to 5.3%, despite a wide range in the age of fish sampled (8–26 years) (Table 1). Variation of Sr:Ca ratios obtained from multiple sampling within a sample site on the otolith was also low, averaging 3.92% ($n = 25$). Accordingly, there was no significant interaction between lake and otolith zone as factors in the ANOVA ($F = 0.15$, df = 2, 28.5, $p = 0.857$). Additional samples taken between the early-growth and edge zones also revealed consistent Sr:Ca ratios across the otolith growth axis among lake trout sampled from different lakes (Fig. 5). Nearest-neighbor discriminant analysis correctly classified 90%–100% of lake trout into their home lake (Table 3).

In sharp contrast with lake trout of known origin, 18 of 20 suspected transplants, ranging in age from 13 to 32 years of age at the time of their collection in 1996 and 1997, exhib-

Fig. 5. Patterns of Sr:Ca ratios along the otolith axes of four known-origin lake trout from (*a*) Lewis Lake (26 years, 949 mm), (*b*) Heart Lake (10 years, 481 mm), (*c*) Heart Lake (15 years, 490 mm), and (*d*) Yellowstone Lake (10 years, 767 mm). Analysis sites were classified by discriminant analysis: ●, Lewis Lake; ▲, Heart Lake; ■, Yellowstone Lake. The dotted lines show the location of annuli and the broken line the otolith edge.

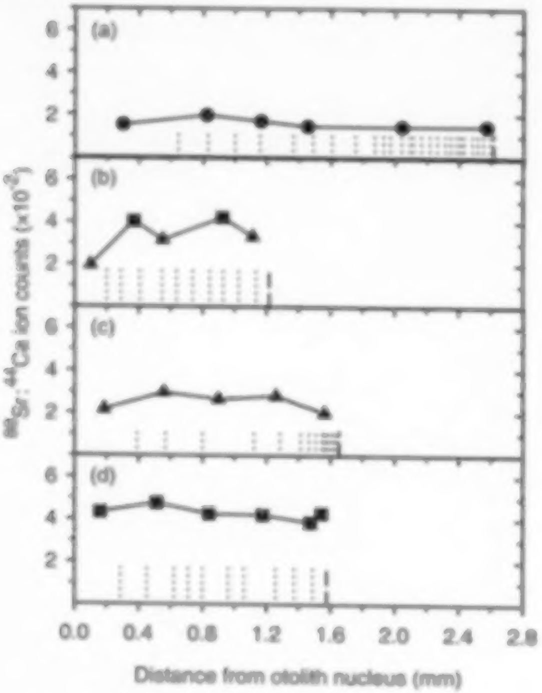
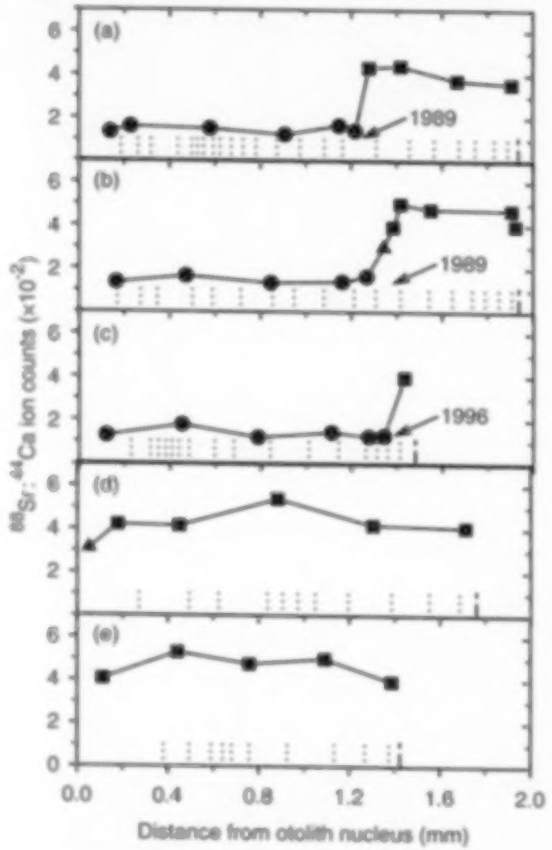


Table 3. Classification of lake trout into probable source lakes.

From lake	% classification into lake		
	Heart	Lewis	Yellowstone
Known-origin			
Heart	100.0	0.0	0.0
Lewis	0.0	90.0	10.0
Yellowstone	0.0	0.0	100.0
Suspected transplants			
Early growth	5.0	90.0	5.0
Edge	20.0	0.0	80.0

Note: Cross-validation results for the known-origin lake trout calibration data set (n = 10 fish per lake) were used to assess classification accuracy. Early-growth and edge zones of the otoliths from the group of suspected transplants captured in Yellowstone Lake (n = 20) were classified into one of the three lakes. The probable origin of lake trout in Yellowstone Lake was based on the early-growth zone Sr:Ca ratios of suspected transplants.

Fig. 6. Patterns of Sr:Ca ratios measured along the otolith axes of five lake trout from the group of suspected transplants collected from Yellowstone Lake: (*a*) 23 years, 832mm, (*b*) 18 years, 850 mm, (*c*) 16 years, 768 mm, (*d*) 11 years, 782 mm, and (*e*) 10 years, 765 mm. Three of the fish show a rapid increase in Sr:Ca ratios corresponding to transplant dates of 1989 (Figs. 6*a* and 6*b*) and 1996 (Fig. 6*c*), whereas the two youngest fish (Figs. 6*d* and 6*e*) show little variation in Sr:Ca ratios, suggesting that they had lived in Yellowstone Lake throughout their lives. Analysis sites were classified by discriminant analysis. ●, Lewis Lake; ▲, Heart Lake; ■, Yellowstone Lake. The dotted lines show the location of annuli and the broken line the otolith edge. Arrows mark the estimated year that the increase in Sr:Ca ratios occurred.



ited substantial increases (mean = 256%) between the early-growth and edge zones (Fig. 4). Eighty percent of the edge zones of suspected transplants were classified by the discriminant model as Yellowstone Lake, whereas 90% of the Sr:Ca ratios measured in the early-growth zone were classified as Lewis Lake (Table 3). This percentage increased to 100% if the two fish that had similar early-growth and edge zone Sr:Ca ratios were excluded from the classification analysis. Sampling along the otolith axis of a random subset (n = 3) of lake trout exhibiting the abrupt shift in Sr:Ca ratio revealed that Sr:Ca ratio increases occurred within a short time period (Fig. 6). Timing of the increase in Sr:Ca ratios was estimated to occur in 1989 for two fish (Figs. 6*a* and 6*b*) and in 1996 for one fish (Fig. 6*c*) of the subset sampled. The other two fish analyzed, representing the lake trout with similar early-growth and edge zone Sr:Ca ratios, showed little variation in Sr:Ca ratios along the otolith axis (Figs. 6*d* and 6*e*). These fish were the youngest fish in the group of suspected transplants (age 10 and 11).

Discussion

Our work demonstrates that otolith chemical composition can be used to identify a probable source and date of exotic species introductions. In the three large lakes that we studied, water chemistry differed significantly among lakes and these differences were directly imparted to lake trout otoliths. The low variation in Sr:Ca ratios of known-origin lake trout along the otolith axis from the early-growth to the edge zone, despite a wide range of ages, established that lake trout from each lake lived in a similar water chemistry throughout their lives. This temporal and spatial stability in otolith chemical signatures was reflected by the high discriminatory power to classify lake trout by their home lake based on unique otolith Sr:Ca ratios. These findings corroborate previous work demonstrating (*i*) a strong association between chemical composition of water and otolith chemistry (Bath et al. 2000; Wells et al. 2003) and (*ii*) that source waters, even in fresh water environments, can be identified with a moderate to high degree of precision based on otolith chemical composition (Thorrold et al. 1998; Wells et al. 2003). Both attributes of otoliths are important in determining stock origins, and our findings indicate that this is particularly relevant for exotic species where stock origin is frequently unknown.

Unlike known-origin lake trout, the large and rapid change in Sr:Ca ratio along the otolith axis of suspected transplants demonstrates that these fish experienced a rapid change in water chemistry. The magnitude of the change in otolith Sr:Ca ratio among suspected transplants (256% increase) mirrors that shown by anadromous fish migrating from fresh water to seawater. For example, Limburg (1995) found that otolith Sr:Ca ratios of age-0 American shad (*Alosa sapidissima*) increased by 250%–620% during movement from fresh water to seawater. Such a large and rapid change in otolith chemistry among lake trout from older year-classes supports the hypothesis that lake trout had been transplanted to Yellowstone Lake. All Yellowstone Lake lake trout from younger age-classes, ≤11 years (1986 estimated year-class and later) at the time of collection in 1996–1999, had similar early-growth and edge zone Sr:Ca ratios, indicating a constant environmental history. In contrast, all lake trout from older year-classes had a marked increase in Sr:Ca ratios between the early-growth and edge zones, indicating that these fish had reared in waters of distinctly different water chemistry during their life-span. These results therefore support the assertion that initial transplanting and natural reproduction of lake trout in Yellowstone Lake likely occurred during the mid- to late 1980s (Kaeding et al. 1996). Although our sample size was not large enough to pinpoint the exact number and timing of transplants, Ruzycki et al.'s (2003) estimate of 298 lake trout >10 years old in 1996 (year-class 1986 and earlier) suggests that a rather large number of individuals were transplanted. Moreover, the dating of the abrupt shifts in otolith chemistry as occurring in 1989 and 1996 suggests that multiple transfers may have occurred.

The classification of 90% of the early-growth zone Sr:Ca ratios of the suspected transplants into Lewis Lake by discriminant analysis suggests that, of the two lakes considered to be the most probable source lakes within Yellowstone National Park, Lewis Lake is the likely source of transplanted lake trout. Lewis Lake, unlike Heart Lake, is accessible by road, which may have facilitated the unauthorized transfer of lake trout into Yellowstone Lake.

Change in Sr:Ca ratios with age or maturation (ontogenetic or physiologic effects) are possible alternatives to the transplant hypothesis. An age- or maturation-induced Sr:Ca ratio increase was unlikely given that lower Sr:Ca ratios would be expected in the early-growth zone among all lake trout. However, the pattern of increased Sr:Ca ratio was only observed in suspected transplants and not in the early-growth zone or among younger age groups of other lake trout sampled from Yellowstone Lake or from any of the lake trout sampled from Heart and Lewis lakes, which varied greatly in age.

Another possible explanation for the increase in Sr:Ca ratios in the otoliths of the suspected transplant group of lake trout is temporal or spatial variation in lake water Sr:Ca ratios of Yellowstone Lake. The large increase in otolith Sr:Ca ratios in 1989 observed in some lake trout from the suspected transplant group coincided with the intense wildfire in Yellowstone National Park in 1988 that altered dissolved ion concentrations of some streams in Yellowstone National Park (Minshall et al. 1997). Although Sr was not measured, other dissolved ions in the lake showed only minor changes in concentration, and Lathrop (1994) and Theriot et al. (1997) found no evidence for significant changes in water chemistry resulting from the 1988 wildfire; therefore, temporal changes in water chemistry seem unlikely to account for the 256% increase in otolith Sr:Ca ratios for Yellowstone Lake lake trout. Yellowstone Lake has many hydrothermal vents that may be a source of local enrichment of Sr and Ca (Balistrieri et al. 2004). However, we found that lake water Sr:Ca ratios varied little (<3.5%) with depth or among lake subbasins; therefore, it is unlikely that the increase in otolith Sr:Ca ratios was a result of fish inhabiting different areas within Yellowstone Lake with different Sr:Ca ratios.

There are two important caveats when assessing the implications of this study. First, the long life-span of lake trout facilitated a long-term retrospective analysis of their environmental history. Detection of unique chemical marks would have been more difficult in species with higher turnover rates or with extensive migrations between waters of differing chemical signatures. Second, although Lewis Lake was identified as the source lake for transplanted lake trout with a high degree of probability, not all ambient waters have unique Sr:Ca signatures (Gillanders et al. 2001; Wells et al. 2003; Munro 2004). Therefore, we cannot eliminate the possibility that lake trout were transplanted from some other (unknown) lake with Sr:Ca ratios similar to those of Lewis Lake. In future studies, use of isotopes or other elements in addition to Sr could enhance the accuracy of identifying source waters (Kennedy et al. 2002; Wells et al. 2003).

There is growing appreciation for just how extensive introductions of exotic species have been and the formidable problem they present for aquatic ecosystem management (Hall and Mills 2000; Rahel 2000; Kolar and Lodge 2002).

For instance, in Montana alone, 375 cases of unauthorized introductions of fishes have been documented in 224 different waters comprising 45 different species (Vashro 1995). Discovery of a new species often poses questions about when the invasion occurred and the geographic origin of the exotic, but thus far, few tools have been available to answer such key questions (McMahon and Bennett 1996; Hebert and Cristescu 2002; Waters et al. 2002). Better knowledge of where exotic species originated and the relative risks they pose is essential for the design of educational and regulatory programs aimed at stemming the tide of future unauthorized introductions (McMahon and Bennett 1996; Kolar and Lodge 2002). Genetic markers have recently been shown to be a useful forensic tool for studying invasion biology (Cristescu et al. 2001; Hebert and Cristescu 2002; Waters et al. 2002). Our study demonstrates how chemical analysis of otoliths can provide a novel forensic tool to estimate geographic origin and timing of exotic fish introductions. Because chemical and genetic analysis techniques each have distinct advantages and limitations (Cristescu et al. 2001; Thorrold et al. 2001; this study), a combination of both tools could provide important new insights into the study of invasion biology.

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Relationship of otolith strontium-to-calcium ratios and salinity: experimental validation for juvenile salmonids

Christian E. Zimmerman

Abstract: Analysis of otolith strontium (Sr) or strontium-to-calcium (Sr:Ca) ratios provides a powerful tool to reconstruct the chronology of migration among salinity environments for diadromous salmonids. Although use of this method has been validated by examination of known individuals and translocation experiments, it has never been validated under controlled experimental conditions. In this study, incorporation of otolith Sr was tested across a range of salinities and resulting levels of ambient Sr and Ca concentrations in juvenile chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*Oncorhynchus kisutch*), sockeye salmon (*Oncorhynchus nerka*), rainbow trout (*Oncorhynchus mykiss*), and Arctic char (*Salvelinus alpinus*). Experimental water was mixed, using stream water and seawater as end members, to create experimental salinities of 0.1, 6.3, 12.7, 18.6, 25.5, and 33.0 psu. Otolith Sr and Sr:Ca ratios were significantly related to salinity for all species (r^2 range: 0.80–0.91) but provide only enough predictive resolution to discriminate among fresh water, brackish water, and saltwater residency. These results validate the use of otolith Sr:Ca ratios to broadly discriminate salinity histories encountered by salmonids but highlight the need for further research concerning the influence of osmoregulation and physiological changes associated with smolting on otolith microchemistry.

Résumé : L'analyse des concentrations de strontium (Sr) et des rapports strontium:calcium (Sr:Ca) dans les otolithes est un outil puissant pour la reconstitution de la chronologie de la migration dans des environnements de salinité différente chez les samonidés diadromes. Bien que la méthode ait été validée par l'examen d'individus connus et des expériences de translocation, elle ne l'a jamais été dans des conditions expérimentales contrôlées. La présente étude vérifie l'incorporation du Sr dans les otolithes sur une gamme de salinités et mesure les concentrations ambiantes de Sr et de Ca qui en résultent chez de jeunes saumons quinnat (*Oncorhynchus tshawytscha*), saumons coho (*Oncorhynchus kisutch*), saumons rouges (*Oncorhynchus nerka*), truites arc-en-ciel (*Oncorhynchus mykiss*) et ombles chevaliers (*Salvelinus alpinus*). L'eau utilisée dans les expérience est un mélange d'eau de ruisseau et d'eau de mer de manière à obtenir des salinités de 0,1, 6,3, 12,7, 18,6, 25,5 et 33,0 ups. Chez toutes les espèces, il y a une relation significative entre la concentration de strontium et le rapport Sr:Ca des otolithes d'une part et la salinité d'autre part (étendue de r^2: 0,80–0,91); cependant, la relation n'est suffisamment prédictive que pour permettre de discriminer entre les séjours en eau douce, en eau saumâtre et en eau salée. Ces résultats permettent de valider l'utilisation des rapports Sr:Ca pour distinguer grossièrement les séjours passés des saumons dans des milieux de différentes salinités; ils démontrent cependant l'importance de recherches futures sur l'influence de l'osmorégulation et des changements physiologiques associés à la transformation en saumoneau sur la microchimie des otolithes.

[Traduit par la Rédaction]

Introduction

Otolith microchemistry is proving to be a powerful tool in reconstructing the chronology of migration by diadromous teleosts among fresh water, estuarine, and saltwater habitats (Campana 1999). Otolith Sr or Sr:Ca ratios have been used to describe migration in many species, ranging from American eel (*Anguilla rostrata*) (Casselman 1982) to striped bass (*Morone saxatilis*) (Secor 1992). Given the occurrence of anadromy within the Salmonidae, the method has been particularly useful in studying migration in this family. For example, otolith Sr or Sr:Ca ratios have been used to describe diadromous migrations in Arctic char (*Salvelinus alpinus*) (Radtke et al. 1996; Babaluk et al. 1997), brown trout (*Salmo trutta*) (Limburg et al. 2001), Atlantic salmon (*Salmo salar*) (Friedland et al. 1998), rainbow trout (*Oncorhynchus mykiss*) (Zimmerman and Reeves 2000), chinook salmon (*Oncorhynchus tshawytscha*) (Zimmerman et al. 2003), and inconnu (*Stenodus leucichthys*) (Howland et al. 2001). In addition to describing migration, increased Sr:Ca ratios in otolith primordia have been used to identify the progeny of anadromous salmonids (Kalish 1990;Volk et al. 2000; Zimmerman and Reeves 2002). Use of otolith microchemistry to reconstruct the chronology of migration among salinity environments, determine the presence of anadromous phenotypes, and identify maternal origin (i.e., anadro-

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mous versus nonanadromous) offers significant opportunities in the study of life history variation and the evolution of diadromous migration in salmonids.

In spite of general acceptance of the use of otolith Sr:Ca ratios to describe migration in salmonids, the method has never been validated through controlled rearing studies under laboratory conditions. Radtke et al. (1996), Limburg et al. (2001), and Zimmerman et al. (2003) used transplantation studies to ensure that otolith Sr:Ca ratios associated with growth in various habitats were as expected (i.e., low in fresh water and higher in salt water). In each case, use of otolith Sr:Ca ratios was supported by the results of the transplantation experiment. In a more direct investigation of the relationship between ambient water chemistry and otolith composition in a salmonid, Wells et al. (2003) compared water chemistry and otolith composition in westslope cutthroat trout (*Oncorhynchus clarki lewisi*) collected from throughout the Coeur d'Alene watershed in northern Idaho and found a linear relationship between water Sr:Ca and otolith Sr:Ca ratios. In that case, Sr:Ca ratios were not meant to be a correlate for salinity but, rather, represented varying ambient water chemistries resulting from variation in lithology and hydrology among watersheds.

In the absence of controlled rearing studies with salmonids, researchers have relied on results from other species. Several studies have examined the relationship of ambient Sr:Ca or salinity and otolith Sr:Ca ratios from fish reared in controlled laboratory conditions. Secor et al. (1995) reared juvenile striped bass at six different salinities ranging from 0 to 30 ppt (in this paper, salinity units are presented as reported in each study and ppt and psu are equivalent). Secor et al. (1995) found a positive linear relationship between salinity and otolith Sr:Ca ratios. Results from wild-caught adult striped bass indicated a logistic relationship between ambient salinity and otolith Sr:Ca ratios in subadult and adult striped bass (Secor et al. 1995). Tzeng (1996) captured elvers of the Japanese eel (*Anguilla japonica*) in an estuary and reared them in the laboratory for 7 months in salinities of 0, 10, 25, and 35 ppt. Mean Sr:Ca ratios in otolith regions associated with the laboratory rearing were highly correlated with salinity. Farrell and Campana (1996) reared Nile tilapia (*Oreochromis niloticus*) in fresh water and artificially enhanced Sr:Ca conditions to compare the incorporation of Sr and Ca from radioisotope-labeled food and water. Strontium and Ca in the otolith were primarily derived from water (through gill uptake), with 75% of Ca and 88% of Sr derived from water rather than from food. It was not clear, however, whether otolith Sr varied directly with Sr availability in the water or was a function of water Sr:Ca ratios (Farrell and Campana 1996). Bath et al. (2000) reared spot (*Leiostomus xanthurus*) in artificial seawater with Sr:Ca ratios corresponding to 1.2×, 1.4×, and 1.8× ambient seawater Sr and Ca levels and found that otolith Sr:Ca ratios were deposited in proportion to Sr:Ca ratios in the ambient water. Kraus and Secor (2003) tested the response of otolith Sr:Ca ratios to variation in salinity (0.5, 5, 10, 15, and 25 psu) and temperature in elvers of American eel. They concluded that broad-scale inference of fresh water, brackish, and ocean migrations was valid, but finer scale estuarine movements among intermediate salinities were not supported by their results. In contrast, Chesney et al. (1998) found no significant relationship between Sr or Sr:Ca ratios and salinity in juvenile gulf menhaden (*Brevoortia patronus*) reared in experimental salinities of 20, 26, and 33.4 psu. Elsdon and Gillanders (2002) tested the interaction of salinity and temperature on otolith Sr:Ca ratios in juvenile black bream (*Acanthopagrus butcheri*) in salinities ranging from 5.5 to 30 ppt and temperatures ranging from 12 to 28 °C. In single-factor experiments, temperature significantly affected otolith Sr:Ca ratios but there was no relationship between salinity and otolith Sr:Ca ratios (Elsdon and Gillanders 2002). In two-factor experiments, however, temperature and salinity interacted to significantly affect otolith Sr:Ca ratios. When considered together, the above studies indicate that use of otolith Sr and Sr:Ca ratios can be an appropriate tool for describing migration across significant salinity gradients.

Because all of the validation studies to date have been conducted on single species, it is difficult to assess the applicability of results for other species. As a result, interspecific variation in Sr incorporation can only be surmised based on comparisons between studies. Secor and Rooker (2000) conducted a metaanalysis of the relationship between otolith Sr:Ca ratios and ambient salinity for 27 species and found a positive relationship. Relatively few intermediate salinity (estuarine) examples were available and Secor and Rooker (2000) suggested that it was "remarkable" that a positive relationship emerged given the diversity of taxa included and range of methods contained in the data set. Ultimately, they suggested that "rigorous empirical testing" is needed to validate the use of otoliths as "faithful environmental chronometers". In this study, I examined the response of otolith Sr:Ca ratios to salinity in five species of anadromous salmonids to calibrate otolith Sr:Ca ratios across the range of salinities typically encountered by juvenile salmonids and assess interspecific variation in otolith Sr and Ca incorporation.

Materials and methods

Juvenile chinook salmon (n = 100), coho salmon (*Oncorhynchus kisutch*) (n = 125), sockeye salmon (*Oncorhynchus nerka*) (n = 125), rainbow trout (n = 125), and Arctic char (n = 125) were obtained from local hatcheries (Alaska Department of Fish and Game, Fort Richardson Hatchery and Cook Inlet Aquaculture Corporation, Trail Lakes Hatchery) and transported to the Alaska Sealife Center in Seward, Alaska. After collection and transportation, all fish were allowed to acclimate in fresh water for at least 7 days prior to initiation of the experiment. Fish were held in an array of six 200-L circular fiberglass tanks with one species in each tank. Fresh water and seawater collected from local sources were used as end members for mixing to produce experimental water representative of the range of salinities encountered in the environment. Fresh water and seawater were continuously supplied from a small stream and from two seawater intake structures located in Resurrection Bay (90 m offshore, 23 m deep), respectively. Fresh water and seawater were continuously mixed and aerated in a 300-L tank and gravity fed to the rearing tanks through a single distribution pipe. Inflow to each rearing tank was 6 L·min^{-1} resulting in a turnover rate of 1.8 times·h^{-1}. Since water was only used once and turned over rapidly, it was assumed that Sr and Ca were continuously replenished and not diluted through uptake as seen in experiments using recirculated water.

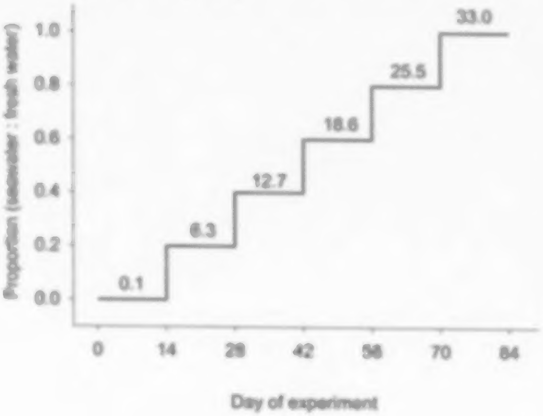
After initiation of the experiment, salinity was increased by proportionally increasing the ratio of seawater to fresh water by 20% every 14 days (Fig. 1) resulting in 14-day experimental salinity periods of 0.1, 6.3, 12.7, 18.6, 25.5, and 33.0 psu. Fish were fed commercial salmon feed (1.2 mm and No. 1 Crumble; Moore-Clark, Vancouver, British Columbia) equal to 5% of fish weight and distributed throughout the day from belt feeders placed above each tank. Mortalities were removed and counted each day.

Water samples were collected from the distribution line at the beginning, middle, and end of each 14-day experimental salinity period. Strontium and Ca concentrations were determined using atomic absorption spectrometry (Laboratory for Oceanographic and Environmental Research, Texas A&M University, Galveston, Texas). Salinity in each tank was monitored on a weekly basis with an electronic salinity meter and temperature was continuously measured in each tank with temperature recording data loggers. To ensure that water samples collected at the distribution line were representative of each rearing tank, I also collected additional samples from all tanks on two occasions (at the midpoint of the 12.7- and 25.5-psu experimental periods). Coefficients of variation were calculated to determine the magnitude of differences in salinity, Ca, Sr, and Sr:Ca molar ratios among tanks.

To identify the beginning of the experiment, I marked otoliths by the immersion of fish in 50 ppm alizarin complexone for 4 h (Thomas et al. 1995; Beckman and Schulz 1996). Between experimental salinity periods, thermal or stress marks were induced on otoliths based on the methods of Letcher and Terrick (1998). Fish were held in 60-L tanks and allowed to warm at least 6 °C before being returned to the rearing tank at the next salinity level. The combination of temperature manipulation and stress associated with increased osmotic demands created a mark evident on the otolith (Fig. 2). Along with the alizarin complexone mark signifying the start of the experiment, the thermal marks were used to guide analysis of otolith microchemistry.

At the conclusion of the experiment, sagittal otoliths were removed from each fish, rinsed, and stored dry in plastic vials. Otoliths, for analysis, were collected from 22 coho salmon, 12 chinook salmon, 12 sockeye salmon, 22 rainbow trout, and 15 Arctic char. Otolith preparation and analyses followed the methods of Zimmerman and Reeves (2000, 2002) and Zimmerman and Nielsen (2003). One sagittal otolith from each fish was mounted sulcus side down with Crystal Bond 509 on a microscope cover slip attached on one edge to a standard microscope slide. The otolith was then ground with 1200-grit sandpaper in the sagittal plane to the level of the nucleus. The mounting medium was heated and the otolith turned sulcus side up. The otolith was then ground with 1200- and 2000-grit sandpaper in the sagittal plane to the level of the primordia and polished with a slurry of 0.05-μm alumina paste. The cover slip was then cut with a scribe so that several prepared otoliths could be mounted on a petrographic slide for microprobe analysis. The slide containing several otoliths was rinsed with deionized water, air dried, and coated with a 400-Å (40-nm) carbon layer.

Fig. 1. Proportion of seawater to fresh water used to create experimental salinity levels and resulting salinity (psu) of inflow to tanks during the rearing study.

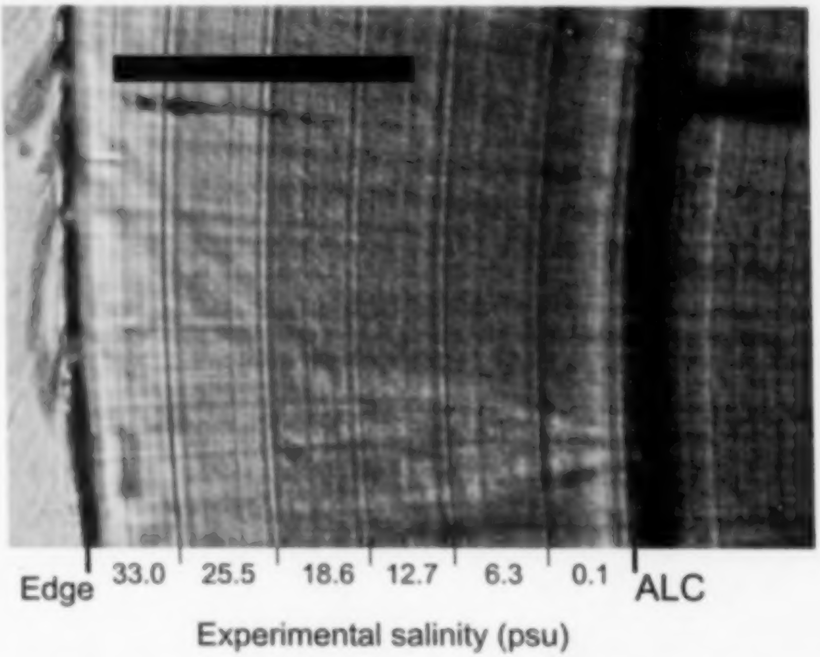


Elemental analysis was conducted with a Cameca SX-50 wavelength dispersive electron microprobe (Cameca, Courbevoie, France). A 15-kV, 50-nA, 7-μm-diameter beam was used for all analyses. Strontiantite and calcite were used as standards for Sr and Ca, respectively. Each element was analyzed simultaneously and a counting time of 40 s was used to maximize precision (Toole and Nielsen 1992). Using the alizarin complexone and thermal marks to guide sampling, Sr and Ca were measured within each experimental growth region (Fig. 2). One point was measured in each experimental region for each fish.

Differences in mean otolith Sr:Ca ratios (atomic ratio), otolith Sr (weight percent), and otolith Ca (weight percent) among treatments were tested by one-way ANOVA and Tukey–Kramer multiple comparisons ($\alpha = 0.05$). With the exception of the rainbow trout treatment, all comparisons were tested using a balanced ANOVA design. Because only four of the 22 rainbow trout analyzed survived to the 33.0-psu salinity treatment, the analysis of this species was unbalanced. The relationship of otolith Sr:Ca ratios to salinity was tested using least-squares linear regression for each species. To examine if Sr and Ca incorporation rates differed among species, I tested homogeneity of the regression lines (slopes) using ANCOVA, and one-way ANOVA was used to test for significant differences in otolith Sr:Ca ratio among species within each salinity treatment. To assess the predictive power of otolith Sr:Ca ratio of salinity, I applied the methods of Prairie (1996) to determine the categorical resolution of otolith Sr:Ca ratio on salinity. This method essentially defines the predictive power of a regression model as the number of separate classes into which the dependent variable can be divided. In addition, the 95% confidence interval of individual measurements was determined to gauge the resolution in terms of salinity units. To compare incorporation rates of otolith Sr among treatments and species, I calculated the distribution coefficient of Sr (D_{Sr}) according to the equation presented by Campana (1999).

$$(1) \qquad D_{\mathrm{Sr}} = \frac{[\mathrm{Sr{:}Ca}]_{\mathrm{otolith}}}{[\mathrm{Sr{:}Ca}]_{\mathrm{water}}}$$

Fig. 2. Photomicrograph of a coho salmon (*Oncorhynchus kisutch*) otolith depicting otolith growth regions associated with experimental salinity levels. ALC indicates the location of the alizarin complexone mark. Scale bar = 100 µm.



This relationship considers the otolith as a calcium carbonate system with dissolved and solid phases and a proportional relationship is expected between the otolith and dissolved Sr in the water (Morse and Bender 1990). The D_{Sr} provides an estimate of rates of strontium incorporation and was calculated to compare with previously published values. A distribution coefficient of 0 would indicate that an element available in the ambient water is not incorporated into the otolith and a value of 1 would indicate that an element is incorporated directly into the otolith with no discrimination (Campana 1999).

The relationship of fish size to otolith Sr:Ca ratio was tested using least-squares linear regression. Fork length at the conclusion of the experiment was tested against otolith Sr:Ca ratio at each salinity level across all species and within each species. Fork length at the conclusion of the experiment was used as a measure of size across all treatments assuming that growth was constant for all fish throughout the experiment.

Results

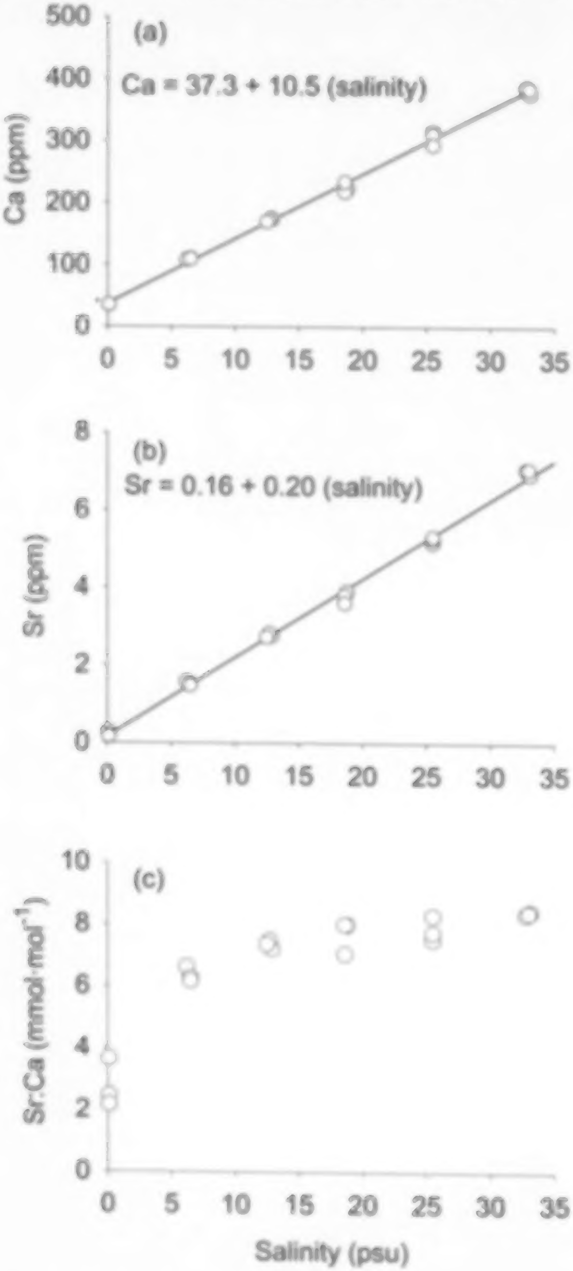
Strontium and Ca were positively correlated with salinity in the experimental mixtures of seawater and fresh water (Ca: $P < 0.0001$; Sr: $P < 0.0001$) (Fig. 3). Ambient Sr ranged from 0.21 to 7.02 ppm and Ca from 35.4 to 386.8 ppm. Although the mixing curves for Sr and Ca were conservative, the Sr:Ca mixing curve was not conservative (Fig. 3). The relationship of Sr concentration and salinity was similar to that presented by Ingram and Sloan (1992) for San Francisco Bay (Sr = 0.051 + 0.23(salinity)) (Fig. 3). Strontium concentrations were highly correlated with those of Ca ($r = 0.99$, $n = 18$, $P < 0.0001$). Similarly, Sr:Ca ratio in water samples was correlated with the concentrations of Ca ($r = 0.83$, $n = 18$, $P < 0.0001$) and Sr ($r = 0.83$, $n = 18$, $P < 0.0001$).

Water temperature in the rearing tanks ranged from 4.86 to 10.74 °C and was positively correlated with the proportion of seawater and hence salinity ($r = 0.85$) (Table 1). On the two occasions when synoptic samples were collected from all five rearing tanks and the inflow, there was little to no variation in salinity, Ca, Sr, and Sr:Ca molar ratios as indicated by low coefficients of variation for each parameter (Table 2). As a result, water samples collected from the inflow appear to represent conditions in each rearing tank. Diel temperature variation was low in the fresh water treatment and increased as the proportion of salt water increased.

Survival to completion of the experiment was 100% in chinook salmon, coho salmon, and sockeye salmon. Survival of rainbow trout was 100% until the 18.8-psu salinity treatment and declined to 2% at the conclusion of the experiment. Similarly, survival of Arctic char was 100% through the 25.5-psu salinity treatment and declined to 65% at the conclusion of the experiment.

Within each species, otolith Sr:Ca ratios increased significantly with ambient salinity (Fig. 4). Mean otolith Sr:Ca ratios were significantly different among salinity treatments within each species (Table 3). As indicated by Tukey–Kramer multiple range tests, there were significant pairwise differences ($P < 0.05$) in otolith Sr:Ca ratios between all treatments in rainbow trout (Fig. 4*d*) and Arctic char (Fig. 4*e*). In chinook salmon (Fig. 4*a*), coho salmon (Fig. 4*b*), and sockeye salmon (Fig. 4*c*), however, mean otolith Sr:Ca ratios were not significantly different among all treatments. Mean otolith Sr:Ca ratios between the otolith growth regions asso-

Fig. 3. (*a*) Ca concentration, (*b*) Sr concentration, and (*c*) Sr:Ca molar ratio in water versus salinity in the inflow to rearing tanks.



ciated with the 12.7- and 18.6-psu growth regions were not significantly different in chinook salmon (Fig. 4*a*) and sockeye salmon (Fig. 4*c*). Similarly, mean otolith Sr:Ca ratios between the otolith growth regions associated with the 18.6- and 25.5-psu growth regions were not significantly different in coho salmon (Fig. 4*b*) and sockeye salmon (Fig. 4*c*). Mean otolith Sr (weight percent) also varied significantly among salinity treatments (Table 3). Tukey–Kramer pairwise comparisons of otolith Sr were identical to those reported for otolith Sr:Ca ratios. Mean otolith Ca (weight percent) did not vary greatly among salinity treatments (Table 3). Only one pairwise comparison of otolith Ca was significant and that was between the fresh water treatment and the 33.0-psu treatment in sockeye salmon.

Table 1. Mean, SD, minimum, and maximum water temperature in rearing tanks during each salinity treatment.

	Experimental salinity period (psu)					
Temperature (°C)	0.1	6.3	12.7	18.6	25.5	33.0
Mean	5.13	5.48	5.76	6.27	7.19	7.79
SD	0.10	0.11	0.17	0.17	0.43	0.93
Minimum	4.86	5.02	5.33	5.95	6.26	6.26
Maximum	5.79	6.72	6.11	6.88	9.96	10.74

Otolith Sr:Ca ratios were positively correlated with salinity in each species: chinook salmon ($r^2 = 0.84$, $n = 77$, $P < 0.0001$), coho salmon ($r^2 = 0.81$, $n = 126$, $P < 0.0001$), sockeye salmon ($r^2 = 0.86$, $n = 73$, $P < 0.0001$), rainbow trout ($r^2 = 0.80$, $n = 109$, $P < 0.0001$), and Arctic char ($r^2 = 0.91$, $n = 90$, $P < 0.0001$). The slopes of these regressions were not homogeneous (ANVOVA test for homogeneity of slopes, $F = 56.10$, $P < 0.001$), indicating that the rate of Sr incorporation varied among species. The predictive resolution (Prairie 1996) of salinity based on otolith Sr:Ca ratios ranged from 2.35 to 2.88 categories, suggesting that, at best, two or three salinity environments can be predicted based on this relationship. Similarly, the 95% confidence intervals for individual prediction ranged from 14 to 18 psu.

Differences among species were suggested by the lack of homogeneity of the slopes of the otolith Sr:Ca ratio to salinity relationship reported above. There were significant differences in otolith Sr:Ca ratios among species in the fresh water treatment (salinity = 0.1; $F_{[4,78]} = 4.45$, $P = 0.005$) and differences in otolith Sr:Ca ratios among species increased as salinity increased: 6.3 salinity ($F_{[4,78]} = 6.01$, $P < 0.001$), 12.7 salinity ($F_{[4,78]} = 18.63$, $P < 0.0001$), 18.6 salinity ($F_{[4,78]} = 36.19$, $P < 0.0001$), 25.5 salinity ($F_{[4,73]} = 50.55$, $P < 0.0001$), and 33.0 salinity ($F_{[4,55]} = 33.92$, $P < 0.0001$).

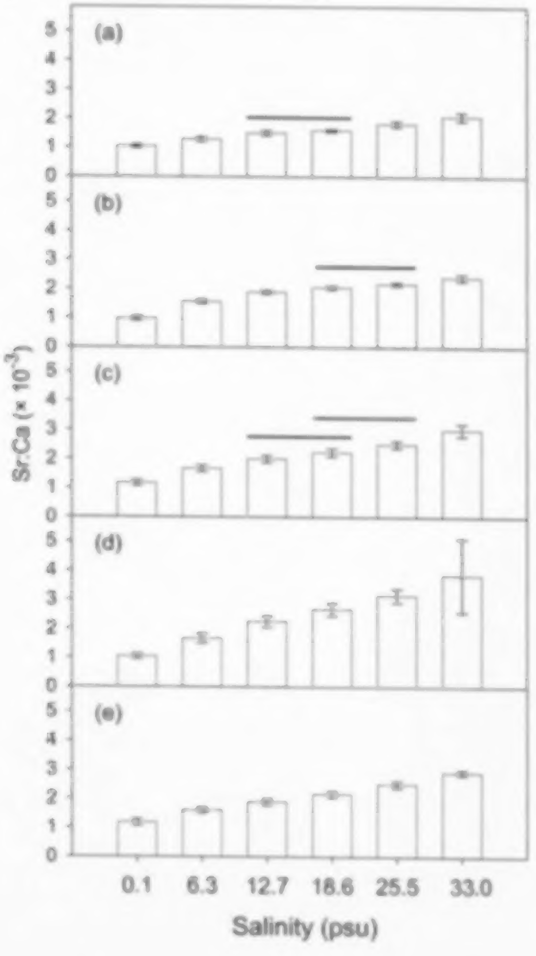
Estimates of D_{Sr} ranged from 0.16 to 0.60 and varied among salinity treatments and among species (Fig. 5). Generally, D_{Sr} was greatest in the fresh water treatment, declined to a minimum in the 6.3-ppt salinity treatment, and gradually increased, thereafter, with increasing salinity. For all species, estimates of D_{Sr} during the fresh water treatment were similar to values reported by Wells et al. (2003) for westslope cutthroat trout in fresh water. For chinook salmon and coho salmon, estimates of D_{Sr} during the salinity treatments were similar to those reported for marine species (Campana 1999; Bath et al. 2000). In the sockeye salmon, Arctic char, and especially rainbow trout treatments, D_{Sr} continually increased with increasing salinity.

Mean fork length of fish varied among species at both the beginning and the end of the experiment (Table 4). Because the species were of different ages, they varied in size at the start of the experiment. The chinook salmon were over 1 year old and the other species were under 1 year old. When all species were pooled, otolith Sr:Ca ratios within

Table 2. Synoptic results of salinity, Ca, Sr, Sr:Ca ratios, and coefficient of variation (CV) in each tank and the inflow measured during the midpoint of the 12.7- and 25.5-psu experimental salinity periods.

	12.7-psu salinity period				25.5-psu salinity period			
	Salinity (psu)	Ca (ppm)	Sr (ppb)	Sr:Ca ratio (mmol·mol^{-1})	Salinity (psu)	Ca (ppm)	Sr (ppb)	Sr:Ca ratio (mmol·mol^{-1})
Inflow	12.7	173	2828	7.49	25.5	312	5273	7.74
Tank 1	12.7	171	2753	7.36	25.5	300	5317	8.11
Tank 2	12.7	172	2823	7.50	25.5	306	5190	7.77
Tank 3	12.7	172	2815	7.49	25.5	312	5204	7.64
Tank 4	12.7	172	2775	7.39	25.5	306	5248	7.85
Tank 5	12.7	172	2802	7.46	25.5	306	5302	7.93
CV (%)	0.0	0.2	1.0	0.84	0.0	1.37	1.08	2.27

Fig. 4. Mean (±SE) otolith Sr:Ca ratios verses ambient salinity of (*a*) chinook salmon (*Oncorhynchus tshawytscha*) (*n* = 12), (*b*) coho salmon (*Oncorhynchus kisutch*) (*n* = 22), (*c*) sockeye salmon (*Oncorhynchus nerka*) (*n* = 12), (*d*) rainbow trout (*Oncorhynchus mykiss*) (*n* = 22), and (*e*) Arctic char (*Salvelinus alpinus*) (*n* = 15). Graphs with lines above the bars indicate similarity between treatments according to Tukey–Kramer multiple range tests.



each salinity treatment (>0.1 psu) were negatively correlated with fork length at the conclusion of the experiment and this relationship became stronger as salinity increased: 0.1 salinity ($r = -0.07$, $n = 74$, $P = 0.55$), 6.3 salinity ($r = -0.32$, $n = 76$, $P < 0.01$), 12.7 salinity ($r = -0.67$, $n = 74$, $P < 0.0001$), 18.6 salinity ($r = -0.77$, $n = 75$, $P < 0.0001$), 25.5 salinity ($r = -0.82$, $n = 73$, $P < 0.0001$), 33.0 salinity ($r = -0.83$, $n = 58$, $P < 0.0001$).

Discussion

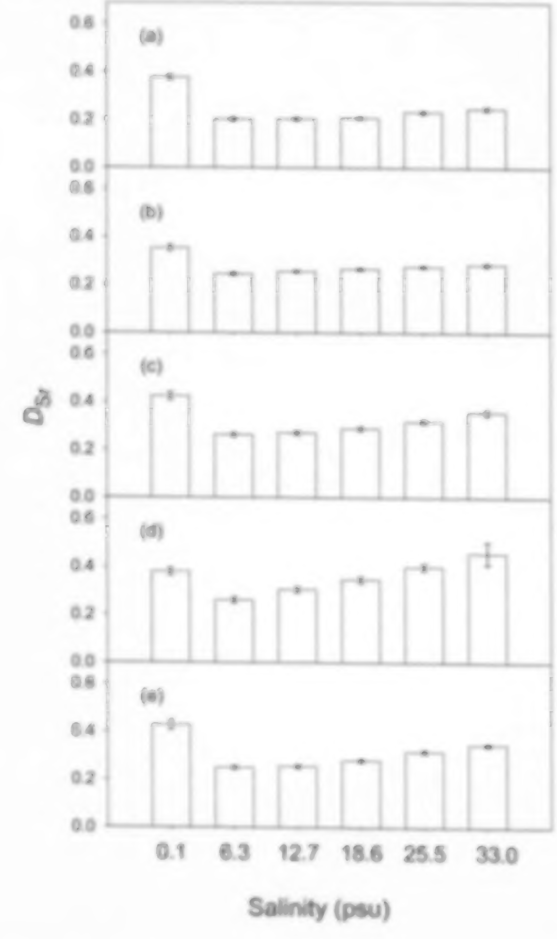
Use of otolith Sr or Sr:Ca ratios to describe migration among fresh water, brackish, and saltwater habitats by salmonids is supported by the results of this study. Otolith Sr and Sr:Ca ratios were linearly related to salinity and hence ambient Sr. Given the magnitude and variability of otolith Sr:Ca ratios observed, however, predictive capabilities provide only enough sensitivity to discriminate between fresh water, brackish water, and seawater. Kraus and Secor (2003) similarly concluded that inference from otolith Sr:Ca ratios was only sensitive enough to discriminate between periods of fresh water and saltwater occupation in elvers of the American eel. Because they reared elvers to approximately 22 ppt salinity, they suggested that extrapolation to 25 or 30 ppt might support discrimination between brackish and ocean occupation. In spite of this lack of sensitivity, the method will remain a powerful tool in the study of diadromous fishes as long as this sensitivity is considered. Depending on the question, this resolution may be adequate to describe migration and aid in the study of sympatric life history types. For example, Zimmerman et al. (2003) were able to confirm marine migration in precocious chinook salmon based on increases in otolith Sr:Ca ratios. Although it would have been beneficial to identify the extent of that migration in terms of salinity, identifying that these fish migrated to salt water at all was significant. More detailed analysis of the range of salinity encountered by these fish would require methods with a greater sensitivity and hence predictive capabilities.

Because this study did not include replicates for each species, it is inappropriate to draw too much from the comparisons among species. It does suggest, however, that there may be differences among species. Although the linear relationship of increasing otolith Sr:Ca ratios with salinity was true for all species, incorporation of Sr varied among even closely related species in the same genus. As a result, it may not be adequate to use the results from other species as reference

Table 3. Summary of ANOVA results for otolith Ca, otolith Sr, and otolith Sr:Ca ratios measured in otolith growth regions associated with experimental salinity treatments.

	Ca (weight %)			Sr (weight %)			Sr:Ca ratio		
Species	*F* ratio	df	*P*	*F* ratio	df	*P*	*F* ratio	df	*P*
Chinook salmon (*Oncorhynchus tshawytscha*)	0.80	5,71	0.55	80.12	5,71	<0.0001	79.91	5,71	<0.0001
Coho salmon (*Oncorhynchus kisutch*)	2.25	5,120	0.05	218.06	5,120	<0.0001	214.35	5,120	<0.0001
Sockeye salmon (*Oncorhynchus nerka*)	2.33	5,67	0.05	94.33	5,67	<0.0001	93.64	5,67	<0.0001
Rainbow trout (*Oncorhynchus mykiss*)	0.76	5,103	0.26	79.53	5,103	<0.0001	79.27	5,103	<0.0001
Arctic char (*Salvelinus alpinus*)	0.87	5,84	0.50	158.60	5,84	<0.0001	168.09	5,84	<0.0001

Fig. 5. Mean (±SE) distribution coefficients of Sr (D_{Sr}) versus salinity for (*a*) chinook salmon (*Oncorhynchus tshawytscha*), (*b*) coho salmon (*Oncorhynchus kisutch*), (*c*) sockeye salmon (*Oncorhynchus nerka*), (*d*) rainbow trout (*Oncorhynchus mykiss*), and (*e*) Arctic char (*Salvelinus alpinus*).



points when determining migrations among salinity environments based on otolith Sr. Howland et al. (2001), for example, suggested that otolith Sr levels observed across otolith transects of inconnu were similar to those measured in anadromous Arctic char held in salt water, thus indicating that the inconnu had migrated to salt water. They warned, however, that rather than suggesting residence in salt water, these results could mean that Sr is incorporated at different rates by these two species. The variation among species observed in this study indicates that this warning is well founded and results from other species should be used as benchmarks with caution.

Although the results from this study followed the expected trend of increasing otolith Sr:Ca ratios with increasing salinity, results from the rainbow trout treatment lead to further questions. For example, the mean otolith Sr:Ca ratios observed in the 12.7-, 18.6-, 25.5-, and 33.0-ppt salinity treatments were all higher than values typically observed in the ocean growth region of wild steelhead (assumed salinity >32 ppt). Mean otolith Sr:Ca ratio in the saltwater growth region of steelhead (C.E. Zimmerman, unpublished data) from the Deschutes River, Oregon, was 0.0021 (SD = 0.0003, *n* = 31), from the Babine River, British Columbia, was 0.0021 (SD = 0.0004, *n* = 20), and from the Saichek River, Kamchatka, was 0.0024 (SD = 0.0003, *n* = 8), whereas in the rainbow trout in this study, mean otolith Sr:Ca ratio in the 25.5-ppt salinity treatment was 0.0031 (SD = 0.002, *n* = 18) and in the 33.0-ppt salinity treatment was 0.0038 (SD = 0.0004, *n* = 4). The reason for this is unclear but may be related to population-specific differences in Sr incorporation at higher salinity or to ontogenetic-related changes in osmoregulatory capabilities. The hatchery population of rainbow trout used in this study was originally founded from a resident population and therefore may exhibit different osmotic capabilities and associated otolith incorporation processes than populations characterized by anadromous phenotypes (steelhead). Boula et al. (2002) showed that even sympatric resident and anadromous brook trout (char) (*Salvelinus fontinalis*) were characterized by physiological differences related to saltwater adaptability. Resident char did not show the same Na^+/K^+ ATPase activity observed in anadromous char and anadromous char were characterized by higher plasma osmolality and Cl and Na concentrations. In this study, rainbow trout experienced increasing mortality as salinity increased in this experiment, suggesting that they were not capable of dealing with the saltwater transition. If the rainbow trout are not capable of dealing with increasing osmoregulatory demands associated with transfer to salt water, changes in blood chemistry and ion balance may result in changes in otolith incorporation rates. In addition, steelhead juveniles typically do not migrate to sea until at least age 1. In this experiment, the rainbow trout were transferred into seawater at least a year earlier, and at a much smaller size than that observed in wild steelhead populations. Since osmo-

Table 4. Mean (±SD) fork length and sample size (in parentheses) for fish at the start and end of the experiment.

Species	Fork length (mm)	
	Start	End
Chinook salmon (*Oncorhynchus tshawytscha*)	114.4±5.3 (34)	130.8±6.5 (13)
Coho salmon (*Oncorhynchus kisutch*)	80.1±4.7 (24)	96.4±7.6 (22)
Sockeye salmon (*Oncorhynchus nerka*)	40.0±4.4 (24)	65.7±4.8 (12)
Rainbow trout (*Oncorhynchus mykiss*)	34.0±3.9 (24)	43.4±5.6 (22)
Arctic char (*Salvelinus alpinus*)	56.0±2.2 (24)	74.8±6.3 (15)

regulation related to smolting is seasonal and related to fish size (Clarke and Hirano 1995), these fish may have been transferred to salt water at an age or size where smolting is not feasible. Further work is needed to elucidate the impacts of smolting and associated changes in osmoregulation and blood chemistry on otolith growth and incorporation of Ca and Sr. For example, calcitonin, a hormone that appears to regulate Ca exchange in gills and bones, increases following the transfer from fresh water to salt water in rainbow trout, during the upstream migration of adult sockeye salmon, and in relation to maturation (Watts et al. 1975; Fouchereau-Peron et al. 1986; Clarke and Hirano 1995). Kalish (1989) hypothesized that seasonal variation in otolith Sr:Ca ratios was largely the result of changes in the proportion of free and bound Ca and Sr present in the blood plasma. Kalish (1989) argued that an increase in Ca-binding proteins, such as albumin or vitellogenin, in the blood would result in reduced Ca in the endolymph and hence in the otolith. Given the potential role of calcitonin in regulating Ca and its fluctuation during the fresh water to marine transition and maturation of salmonids, its relationship to otolith formation should be investigated.

Along with the findings of Wells et al. (2003), this study suggests that further work is needed to examine the kinetics of Sr incorporation in fresh water and diadromous species. The D_{Sr} values for all species in the fresh water treatment of this study were similarly high and comparable with that reported by Wells et al. (2003) for cutthroat trout in freshwater. The D_{Sr} values associated with the salinity treatments, however, were similar to values reported for marine fishes (Kalish 1989, 1991; Bath et al. 2000). However, D_{Sr} values were consistently high in rainbow trout, Arctic char, and sockeye salmon across the range of salinity treatments. The D_{Sr} values were similar among species at the 6.3-ppt salinity treatment but diverged as salinity increased. The D_{Sr} in chinook salmon was similar to values reported for marine fishes across the range of salinity treatments. The D_{Sr} in rainbow trout, however, steadily increased as salinity increased. Similarly, Arctic char and sockeye salmon exhibited higher than expected D_{Sr} as salinity increased. Perhaps increased D_{Sr} in these cases reflects ontogenetic variation in seawater adaptation or population-level differences in seawater adaptability as discussed above. Wells et al. (2003) suggested that the elevated value observed in fresh water cutthroat trout reflected the difference between Sr and Ca uptake in fresh water versus marine environments. They suggested that this difference was the result of the different pathway of elemental uptake between marine and fresh water environments (through intestinal walls for marine fishes and through chloride cells in the gills for fresh water fishes) (Wells et al. 2003).

Geographic and seasonal variation of ambient Sr in fresh waters should be considered in all studies examining migration among salinity environments. Ambient concentrations of Sr and Ca should be determined among the habitats to be studied (Rieman et al. 1994; Wells et al. 2003; Zimmerman et al. 2003). Given the potential variability of ambient Sr resulting from differing lithology and hydrology, it is critical that analysis of ambient water chemistry be a part of all studies using otolith Sr:Ca ratios to reconstruct movements among salinity environments to ensure that fresh waters conform to the assumption of low Sr. Several questions remain concerning the application of otolith Sr and Sr:Ca ratios in the study of fresh water and marine migrations of salmonids. These questions include uncertainty concerning the role of smolting and maturation on incorporation of Ca and Sr in otoliths. This is especially critical, since these are processes occurring as fish make the transition between salinity environments (the very thing that we are seeking to identify using otolith Sr:Ca ratios). In addition, the roles of temperature (Elsdon and Gillanders 2002) and dietary Sr are still unresolved.

In spite of limited resolution in terms of predicting salinity, otolith Sr concentrations or Sr:Ca ratios provide an important tool in the study of migration in diadromous salmonids. Continued utility of the method, however, will require further refinement, validation, and research concerning the interactive effects of ambient water chemistry, temperature, and physiology.

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A long-term, spatially replicated experimental test of the effect of marine reserves on local fish yields

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Abstract: Do no-take marine reserves affect fishery yields? Manipulations of reserve status, and yield estimates, were made at two Philippine islands over two decades. Twenty-five percent and ten percent, respectively, of the coral reefs at Sumilon and Apo islands were made no-take reserves in 1974 and 1982. Biomass of target fish increased inside the no-take reserves 3- to 4.5-fold over 9–18 years. Biomass did not increase outside each reserve. Protection of the Sumilon reserve ceased in 1984. Biomass of targeted fish in the reserve and trap and gillnet catches of these fish declined by 42.7% and 40%, respectively, by 1985. The reserve was reprotected from 1987 to 1991 and from 1995 to 2001. Fish biomass increased in the reserve by 27.2%. Trap and gillnet catches outside the reserve increased 26.9% by 2001. The Apo reserve was protected from 1982 to 2001. Total catch of major fish families was significantly higher after (1985–2001) than before (1981) reserve establishment at Apo, increasing 41.3% between 1981 and 1998–2001. These experiments, plus spillover evidence, suggest that marine reserves may help maintain, or even enhance, local fishery yields in the long-term.

Résumé : Est-ce que les réserves marines sans pêche affectent le rendement des pêches? Des manipulations du statut de réserves et des estimations des rendements ont été faites près de deux îles des Philippines au cours de deux décennies. Respectivement 25 % et 10 % des récifs coralliens près des îles Sumilon et Apo ont été déclarés des réserves sans pêche en 1974 et en 1982. La biomasse des poissons ciblés a augmenté d'un facteur de 3–4,5 fois dans les réserves sans pêche au cours d'une période de 9–18 ans. La biomasse n'a pas augmenté à l'extérieur de ces deux réserves. La protection de la réserve de Sumilon a cessé en 1984. En 1985 dans la réserve, la biomasse des poissons ciblés et les captures de ces poissons dans des pièges et les filets maillants avaient diminué respectivement de 42,7 % et de 40 %. La réserve a bénéficié de nouveau de protection en 1987–1991 et en 1995–2001. La biomasse des poissons dans la réserve a augmenté de 27,2 %. En 2001, les captures au piège et au filet maillant hors de la réserve avaient augmenté de 26,9 %. Les captures totales de poissons des familles importantes étaient plus fortes après (1985–2001) qu'avant (1981) l'établissement de la réserve à Apo et ont augmenté de 41,3 % entre 1981 et 1998–2001. Ces expériences, plus des évidences de l'existence de débordements de populations, indiquent que les réserves marines peuvent servir à maintenir et même à améliorer à long terme les rendements des pêches locales.

[Traduit par la Rédaction]

Introduction

No-take marine reserves are areas of the marine environment in which all forms of extraction by humans, primarily fisheries, are banned permanently (Allison et al. 1998; Roberts and Hawkins 2000; Gell and Roberts 2002). Marine reserves are often promoted as a mechanism to address problems such as loss of marine biodiversity (Dayton et al. 2000; Roberts and Hawkins 2000; Jackson et al. 2001) and alteration of trophic structure of marine ecosystems (Pauly et al. 1998; Jackson et al. 2001). They are also often promoted as one tool to help manage fisheries (Gell and Roberts 2002; Russ 2002; Gerber et al. 2003). In addition, marine reserves have other important economic and social benefits, such as enhancing tourism (Roberts and Hawkins 2000).

A major expectation of no-take marine reserves as fisheries management tools is that they will, in the long-term, allow buildup of spawning stock biomass within them and help maintain, or even enhance, fishery yields outside them (Alcala and Russ 1990; Gell and Roberts 2002). Marine reserves are expected to sustain fisheries external to them by becoming net exporters of adults (the spillover effect) and net exporters of propagules (the recruitment effect) (Russ 2002). The spillover effect is generally expected to operate locally on spatial scales of hundreds of metres to kilometres for many organisms targeted by fisheries (McClanahan and

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Mangi 2000; Roberts et al. 2001; Russ 2002). The recruitment effect is expected to operate on the scales of dispersal of pelagic larvae, perhaps tens of kilometres (Palumbi 2001; James et al. 2002; Gaines et al. 2003). Local fishery benefits from spillover (net export of adults) and possible localized export of propagules will likely generate support from fishing communities for marine reserves (Russ and Alcala 1996; Roberts et al. 2001; Gell and Roberts 2002). However, long-term experimental tests of the effects of marine reserves on local fishery yields are rare.

There is a rapidly increasing number of empirical and theoretical studies suggesting that marine reserves can affect fisheries in a positive way beyond their boundaries (Gell and Roberts 2002). The majority of this evidence consists of studies demonstrating changes in abundance of targeted species or fishing patterns outside the boundaries of no-take reserves. Such changes include higher abundance (Rakitin and Kramer 1996; Russ and Alcala 1996), fisheries catch per unit effort (CPUE) (Rakitin and Kramer 1996; McClanahan and Kaunda-Arara 1996; McClanahan and Mangi 2000), and fishing effort (Murawski et al. 2000; Kelly et al. 2002) close to, but not farther from, reserve boundaries. Other studies have demonstrated development of higher CPUE over time outside reserves (McClanahan and Kaunda-Arara 1996; Roberts et al. 2001; Maypa et al. 2002). However, there are few studies that examine the key question of the effect of no-take reserves on absolute yields of the fishery (Alcala and Russ 1990; McClanahan and Kaunda-Arara 1996; Roberts et al. 2001).

A common expectation of fishers is a loss of fisheries yield following reserve establishment, approximately equivalent to that caught previously in the closed area (Roberts and Hawkins 2000). This assumes that total fishing effort stays the same after implementation of the reserve. Some fishers even suggest that total fishery yields may decline, since the remaining fishing effort is concentrated on the stocks outside the reserve, causing overfishing. However, if biomass of targeted fish can build up over time inside the reserve, leading to spillover, and perhaps localized export of propagules, the total loss of fisheries yield may not equal what was caught previously in the area before it became a reserve. Furthermore, fishing patterns may change because of other effects of reserves, for example increased income to the fishing community generated from tourism by the reserve (e.g., Russ et al. 2004). In such cases, absolute yields may stay stable (Maypa et al. 2002) or even improve (Russ et al. 2004). A neutral or positive effect of a reserve on absolute yield may occur provided that sufficient time is available to allow substantial recovery of fish stocks both inside and outside the reserve.

The effect of establishment of no-take reserves on fisheries yields, be it negative, neutral, or positive, will clearly be dependent on the spatial and temporal scales of study. At the spatial scale of the entire stock, loss of catch owing to loss of fishing area has to be balanced against any gains owing to the spillover and recruitment effects. Clearly, the balance between loss and gain of yield will vary over time, as biomass builds up over time in the reserves relative to the fished areas. At the scale of a local fishery, it has been assumed until recently that loss of catch owing to loss of fishing area had to be balanced against any gains owing only to spillover. However, recent studies of the potential for limited larval dispersal and "self-recruitment" (e.g., Jones et al. 1999; Swearer et al. 1999; James et al. 2002) have led to the possibility that reserves may affect local fishery yields by both spillover and the recruitment effect. Gell and Roberts (2002) recently suggested that abundance and catch rates outside reserves in St. Lucia (Roberts et al. 2001), the Egyptian Red Sea (Galal et al. 2002), and the Philippines (Maypa et al. 2002) have improved so markedly that the local fisheries may have been affected by larval export as well as spillover.

In this study, we investigate the effects of experimental manipulations of marine reserve status on local fishery yields at two small islands in the Philippines, Sumilon and Apo. These studies have been carried out over periods of 22 and 20 years, respectively. We ask the question does establishment of a no-take marine reserve have a negative, neutral, or positive effect on local fishery yield in the long term?

Materials and methods

Study sites

The study was carried out at two islands in the central Philippines (Fig. 1). Sumilon Island southeast of Cebu (9°21′N, 123°23′E) and Apo Island southeast of Negros (9°4′N, 123°16′E). Sumilon Island had a no-take marine reserve established on its western side in December 1974. Apo Island had a no-take marine reserve established on its southeastern side in late 1982. Sumilon Island is a coralline island of 0.23 km^2 surrounded by a fringing coral reef of 0.5 km^2 to the 40-m isobath. Apo Island is a mainland island of 0.7 km^2 surrounded by 1.06 km^2 of fringing coral reef to the 60-m isobath (0.7 km^2 to the 20-m isobath).

The Sumilon reserve is a 0.75-km section (approximately 25% of the coral reef area) of the western side of the island (Fig. 1). The area of the reserve to 500 m from shore is 37.5 ha. The Apo reserve is a 0.45-km section (approximately 10% of the coral reef area) of the southeastern side of the island (Fig. 1). The management histories of the Sumilon and Apo reserves and the fished portions of these two islands are provided in Table 1. The Sumilon reserve has had a complex history of management over the period 1974–2001 (Table 1) (Russ and Alcala 1999). The no-take status of the Apo marine reserve has been maintained successfully from 1982 to 2001 (Table 1) (Russ and Alcala 1999). Decisions to apply no-take status at each island and to remove it at Sumilon were taken by the local municipalities. In all cases, these decisions were taken because local leaders perceived that the actions would benefit the local communities. For example, no-take status was applied at Sumilon (1974 and 1987) and Apo (1982) in the hope of maintaining fisheries and enhancing tourism at the islands. No-take status was removed at Sumilon (1984) when new mayors were elected that did not support the reserve concept (Russ and Alcala 1999). Thus, the management histories were not designed as scientific experiments. However, they are natural experiments in the sense that management actions did result in real changes to no-take status.

Method of visual census

Estimates of fish biomass were made visually at the reserve and a nonreserve (fished) site at each island in Decem-

Fig. 1. Map showing the location of Sumilon and Apo islands, central Philippines, and the location of the unfished reserve areas (shaded) and the fished nonreserve areas at each island. The positions of the six 50 m × 20 m replicate underwater visual census plots (solid rectangles) surveyed at each sampling site and time are shown.

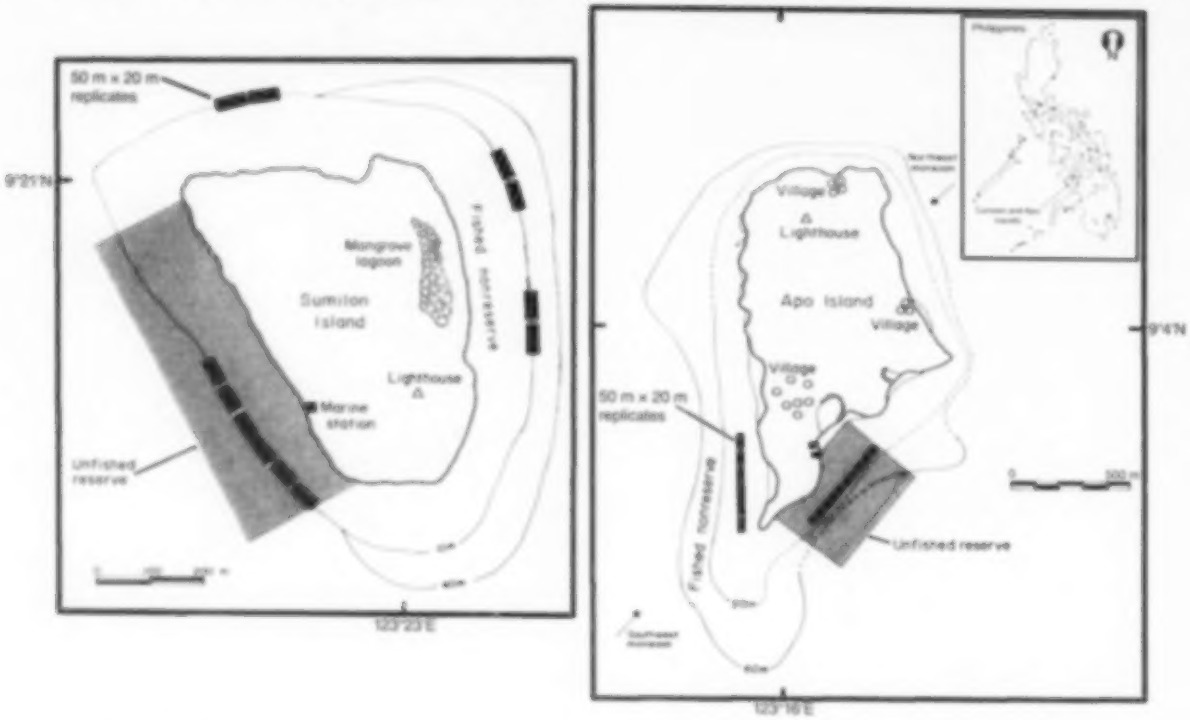

50 m × 20 m replicates
9°21'N
Mangrove lagoon
Sumilon Island
fished nonreserve
Lighthouse
Marine station
Unfished reserve
0 100 200 m
20m
40m
123°23'E
Philippines
N
Northeast monsoon
Village
Lighthouse
Apo Island
Village
9°4'N
Village
50 m × 20 m replicates
Fished nonreserve
Unfished reserve
0 500 m
20m
40m
Southwest monsoon
123°16'E

Table 1. History of protection status for the two no-take reserves and control (fished) portions (nonreserves) at Sumilon and Apo islands over the period 1974–2001.

Site	Time period	Protection status	Comments
SR	Dec. 1974 – May 1984	No-take for 9.5 years	No-take status established under a municipal law and maintained by caretaker
SR	1984–1986	Reserve pulse-fished for about 2.5 years	Caused by election of new municipal mayors. Fishing techniques included explosives and drive nets ("muro-ami")
SR	1987–1991	No-take for 5 years	No-take status reestablished by local municipality
SR	1992–1994	Reserve opened to fishing	~100 municipal fishers using traps, hook and line, gill net, and spear
SR	1995–2001	Fishing restricted	All fishing except hook and line banned
SNR	1974–1987	Open to fishing	~100 municipal fishers using traps, hook and line, gill net, and spear
SNR	1987–1991	No-take for 5 years	No-take status established by local municipality
SNR	1992–2001	Open to fishing	~100 municipal fishers using traps, hook and line, gill net, and spear
AR	Dec. 1982 – Dec. 2001	No-take for 19 years	No-take status established and maintained by local community (~500 residents)
ANR	1982–2001	Open to fishing	Nonreserve area fished by ~200 fishers using traps, hook and line, gill net, and spear
AR, ANR	Nov. 1986	Marine management plan	Marine management plan established under municipal law. This banned explosives, drive nets, small-mesh nets, poisons from the entire island, and banned fishing by nonresidents

Note: S, Sumilon Island; A, Apo Island; R, reserve; NR, nonreserve. Histories derived from Russ and Alcala (1999) and White et al. (2002).

ber or November of each year from 1983 to 2001 except for 1984, 1986–1987, and 1996. Five families of reef fish (Acanthuridae, Carangidae, Lutjanidae, Lethrinidae, and Caesionidae) were censused. These families made up 92.3% and 82.8% of the fisheries yield at Sumilon and Apo, respectively (Alcala 1981; Alcala and Russ 1990; Maypa et al. 2002). The method of underwater visual census has been published elsewhere (Russ and Alcala 1998). Six 1000-m^2 replicate areas of reef slope were censused in the reserves (2–17 m depth) and nonreserves (9–17 m depth) on each sampling occasion. The observer (G.R.R.), the method of underwater visual census, and the position of the replicates were the same from 1983 to 2001 (Fig. 1) (except that some replicate areas at the Apo nonreserve site differed between 1983 and all other times). The abundances of small species of Acanthuridae (*Ctenochaetus*, small *Acanthurus*, and small *Naso*) and all species of Caesionidae were estimated cumulatively in log 4 abundance categories in a census area. These categories were as follows: category 1 = 1 fish, category 2 = 2–4 fish, category 3 = 5–16 fish, category 4 = 17–64 fish, category 5 = 65–256 fish, category 6 = 257–1024 fish, and category 7 = 1025–4096 fish. Use of these abundance categories is likely to compromise accuracy of estimates for speed of surveys. However, any potential bias in biomass estimates will be likely consistent across times and sites. Simultaneously, actual counts and estimates of total length (±5 cm) of large Acanthuridae (large *Acanthurus* and large *Naso*) and all species of Carangidae, Lutjanidae, and Lethrinidae were made. Juveniles (<10 cm) were not counted. An estimate of biomass for those species for which density and size structure data were available was constructed using published length–weight relationships for the species or closely related species (Froese and Pauly 1997). Length estimates were not made for Caesionidae and individuals of small species of Acanthuridae. These species thus lacked size structure data. An estimate of average length of a fish in the genus was made and converted to an average weight from published length–weight relationships (Froese and Pauly 1997). Abundances of Caesionidae and the small species of Acanthuridae were converted to approximate estimates of density by using midpoints of abundance categories. Density and average weight estimates were then used to estimate biomass per unit area. A long-term and substantial decline in abundance of Caesionidae occurred in the Apo Reserve beginning around 1992, possibly associated with reduced recruitment (Russ and Alcala 1998). Thus, we express trends in biomass and catch at Apo with and without this family.

Estimates of annual catch

Annual trap and gillnet catch of five families (Acanthuridae, Carangidae, Lutjanidae, Lethrinidae, and Caesionidae) was estimated in five separate years at Sumilon Island (Table 2). The years 1979, 1980, and 1983 were years of relatively long-term reserve protection (6, 7, and 10 years) at Sumilon. In 1985–1986, the reserve had been pulse-fished for almost 2 years, and fish biomass in the reserve had been reduced significantly (Alcala and Russ 1990). By 2001, the reserve had been reprotected to varying degrees since 1987, and biomass had shown some degree of recovery. Annual catch of trap, gill net, hook and line, and spear of the same five families was estimated at Apo once before (1980–1981: Alcala and Luchavez 1981) and five times after (1985–1986, 1986, 1997–1998, 2000, and 2001: White and Savina 1987; Bellwood 1988; Maypa et al. 2002) reserve establishment (Table 2). Catch data at Sumilon and in the early studies at Apo were recorded at the family level. Although each family contains many targeted species, such pooling within families is justified, since life histories are generally consistent within each family, with the possible exception of the Acanthuridae.

Data analysis

Statistical comparisons of biomass of fish at the reserve and nonreserve sites over the 14 times were made separately for each island using univariate, repeated-measures ANOVA. The three factors in these analyses were reserve (two levels), plots (six levels) nested within each reserve and nonreserve site (a random factor), and the repeated measure time with 14 levels. The data were examined for homogeneity of variance (Cochran's test at $p < 0.05$) (Underwood 1981), skewness and outliers (Box plots), normality, and correlations between means and variances. Data were transformed ($\log_{10}(x)$). We used Greenhouse–Geisser adjusted p values for the reserve–time interaction (Statsoft, Inc. 1995). Temporal trends in biomass at reserve and nonreserve sites at each island were examined by linear regression. Biomass was plotted against years of reserve protection for both the Sumilon and Apo reserves. One of the 14 sampling times (1992) at the Sumilon reserve was omitted from these regressions. In 1992, the Sumilon reserve had been open to fishing for 1 year (and thus, years of protection was assigned a value of –1) but had built up considerable fish biomass during 5 years of protection from 1987 to 1991 (Table 1). Fish biomass in the nonreserve sites was plotted against years of reserve protection at the island by simply plotting the nonreserve biomass for the same year as that for the biomass estimate in the reserve for any given duration of protection. Four of the 14 sampling times (1988, 1990, 1991, and 1992) at the Sumilon nonreserve were omitted from these regressions. During this time, the Sumilon nonreserve was protected from fishing (1987–1991) or had been open to fishing for a short time following a long period of no fishing (Table 1).

A one-sample t test was used to compare trap plus gillnet catch of the five families at Sumilon in 1985 (after the reserve biomass was fished down) with that over the period 1979–1983 (6–10 years of reserve protection). The same test was used to compare trap plus gillnet catch of the five families at Sumilon in 2001 (after the reserve biomass had recovered somewhat) with that over the period 1979–1983 (6–10 years of reserve protection). A one-sample t test was used to compare trap, gillnet, hook and line, and spear catch of four families at Apo (minus Caesionidae) in 1980–1981 (before reserve establishment) with that over the period 1985–2001 (3–18 years of reserve protection). Biomasses of the five families of reef fish in the Sumilon reserve were compared between 1983 (protected for 10 years) and 1985 (fished down) and between 1985 (fished down) and 2001 (some level of recovery) with t tests. Changes in catch with duration of reserve protection at each island were examined by linear regression.

Table 2. Details of estimates of annual catch of Acanthuridae, Carangidae, Lutjanidae, Lethrinidae, and Caesionidae at Sumilon Island between 1979 an 2001 and Apo Island between 1980–1981 and 2001.

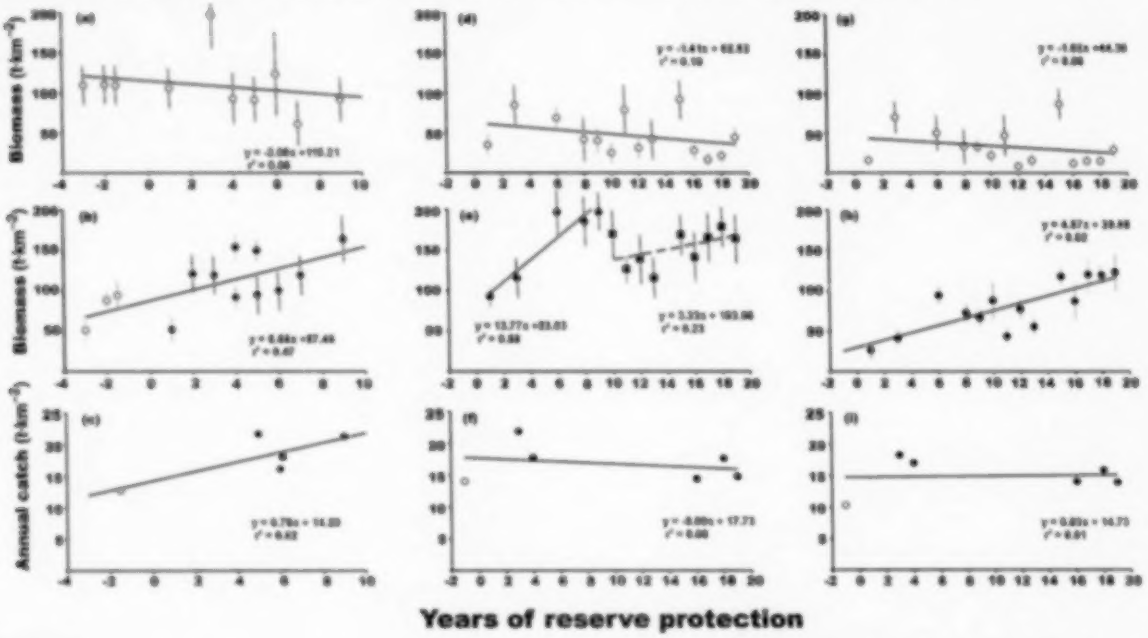
Island	Study	Period	Before or after reserve	Areas surveyed	Methods
Sumilon	Alcala 1981	Jan.–Dec. 1979 and 1980	After	Whole reef	A caretaker on the island estimated trap and gillnet catch. He recorded fish type caught by gear and fish weights (estimated to ±10%) 6–7 days per month in each year. The one day each week when all traps were lifted was always sampled plus days selected haphazardly for observations and interviews
Sumilon	Alcala and Russ 1990	Jan.–Dec. 1983 and Dec. 1985 – Dec. 1986	Long-term protection (1983), fished down (1985–1986)	Whole reef	As above for 1983. The 1985–1986 catch of traps and gill nets was estimated as above by a new assistant (an experienced fisher trained in the estimation of weights of catch). He was not an island resident. Catch was sampled 4–5 days per month (including at least 2 days when traps were lifted)
Sumilon	A.C. Alcala, unpublished data	Jan.–Dec. 2001	After	Whole reef	Two trained fisher assistants recorded weights of fish caught by gill nets and hook and line (combined) every day and by traps on Fridays when the traps were lifted. Local names of fish were assigned to families. To estimate the 2001 gillnet catch, we assumed that the proportion of gillnet to hook and line catch was the same in 2001 as the mean for 1983 and 1985
Apo	Alcala and Luchavez 1981	May 1980 – March 1981[a]	Before	Fish market, landing sites	Questionnaires and interviews with fishers and direct observations. Forty-nine days of surveys, most days market day (Wednesday). Type of fish, gear used, weight of catch
Apo	White and Savina 1987	April 1985 – March 1986	After	Landing sites	Daily estimates made by seven fish dealers – volunteers. Type of fish, gear used, weight of catch
Apo	Bellwood 1988	Jan.–Dec. 1986	After	Fish market	Every market day (Wednesday). Researcher recorded species of fish, gear used, weight of catch. Data converted to annual estimates
Apo	H.P. Calumpong, unpublished data	June 1997 – Sept. 1998[a]	After	Three main landing sites	Daily creel surveys by research assistant. Species of fish, gear used, weight of catch[b]
Apo	Maypa et al. 2002	Jan.–Dec. 2000	After	Three main landing sites	Creel surveys (three randomly chosen days per week converted to weekly estimate) by researcher or assistant. Species of fish, gear used, weight of catch (weighing scales)[b]
Apo	Maypa et al. 2002	Jan.–Dec. 2001	After	Three main landing sites	As above for 2000

Note: Catch at Sumilon is trap and gillnet catch (gears that account for 64% of catch at the island). Hook and line catch was not estimated at Sumilon until 1983 and thus was omitted from the comparisons. Catch at Apo is hook and line, trap, gill net and spear (gears that account for 100% of the catch at the island).

[a] Extrapolated to annual estimate.

[b] At Apo Island, fishers land their catch in many different sites, making it difficult to record every fish landing. Three major landing sites were monitored in 1997–1998, 2000, and 2001. The estimated annual catch for these years was adjusted by adding 10% to the actual values for both reef-associated and nonreef catches to account for this. This figure of 10% was based on the mean estimates given by fishers through interviews in 2000 (mean = 10.2, SE = 0.29, n = 55) and 2001 (mean = 10.5, SE = 0.41, n = 55) as the amount of catch not recorded in surveys.

Fig. 2. Underwater visual census estimates of biomass of five families of reef fish (Acanthuridae, Carangidae, Lutjanidae, Lethrinidae, and Caesionidae) in reserve and nonreserve sites (1983–2001) and estimates of total annual catch of these families (1979–2001) plotted against years of reserve protection at Sumilon and Apo islands. Negative years of protection at the Sumilon reserve indicate years open to fishing. Open data points are biomass estimates when the site was open to fishing or catch estimate at the island in the absence of reserve. Solid data points are biomass estimates when the site was closed to fishing or catch estimate at the island in the presence of reserve. Shaded data points are biomass or catch estimates when the Sumilon reserve was partially closed to fishing. Error bars for biomass estimates are SEs. (*a*–*c*) nonreserve biomass, reserve biomass, and catch at Sumilon; (*d*–*f*) nonreserve biomass, reserve biomass, and catch at Apo; (*g*–*i*) nonreserve biomass, reserve biomass, and catch at Apo with Caesionidae omitted.



Results

The biomass of targeted fish (Acanthuridae, Carangidae, Lutjanidae, Lethrinidae, and Caesionidae) had a significant positive correlation with years of reserve protection inside ($F_{[1,11]} = 9.77$, $p < 0.01$) but not outside ($F_{[1,8]} = 0.48$, $p > 0.05$) the Sumilon reserve (Figs. 2*a* and 2*b*) (repeated-measures ANOVA: protection–time interaction, $F_{[13,130]} = 3.30$, $p < 0.001$) (Table 3). The biomass of targeted fish increased by a factor of 3.1 between 1 and 9 years of reserve protection at Sumilon (Fig. 2*b*). Fish biomass was relatively high and variable in abundance in the Sumilon nonreserve (Fig. 2*a*). However, a clear difference in the fish biomass between reserve and nonreserve sites was apparent at around 6 years of reserve protection (Figs. 2*a* and 2*b*). The biomass of the same five fish families had a significant positive correlation with years of reserve protection inside the Apo reserve for the first 9 years of protection ($F_{[1,3]} = 21.02$, $p < 0.05$) (Fig. 2*e*) but not for the next 9 years ($F_{[1,7]} = 2.10$, $p > 0.05$) (Fig. 2*e*). There was no significant correlation between years of reserve protection and biomass of these five families in the Apo nonreserve ($F_{[1,12]} = 1.34$, $p > 0.05$) (Fig. 2*d*). The biomass of the same five fish families minus Caesionidae did have a significant positive correlation with years of reserve protection inside ($F_{[1,12]} = 19.88$, $p < 0.001$) but not outside ($F_{[1,12]} = 0.72$, $p > 0.05$) the Apo reserve over the full 18 years of the study (Figs. 2*g* and 2*h*) (repeated-measures ANOVA: protection–time interaction, $F_{[13,130]} = 4.56$, $p < 0.001$) (Table 3). The biomass of targeted fish increased by a factor of 4.6 between 1 and 19 years of reserve protection at Apo (Fig. 2*h*). A clear difference in the fish biomass between reserve and nonreserve sites was apparent at around 6 years of reserve protection (Figs. 2*g* and 2*h*). The results at both islands are consistent with the hypothesis that the removal of fishing caused the increase in fish biomass in the reserves.

The correlations between biomass of the five families and reserve protection reported for Sumilon Island (Fig. 2) resulted from a complex history of management at the island (Russ and Alcala 1999). The effects of this history on the fish biomass in the Sumilon reserve and nonreserve for the period 1983–2001 are also shown (Fig. 3). In the Sumilon reserve, fish biomass declined by 42.7% between 1983 and 1985 during an intense fishing event (Fig. 3*a*; Table 1). Biomass rose in the reserve 59.6% by 1991 after 5 years of protection (Fig. 3*a*; Table 1) and then fell 65.8% by 1994 after the reserve was again opened to fishing (Fig. 3*a*; Table 1). When all forms of fishing except hook and line were banned in the reserve in 1995, biomass rose by 132% by 2001 (Fig. 3*a*; Table 1). Biomass in the reserve rose by 26.7% between 1985 and 2001 during this period of intermittent protection (Fig. 3*a*; Table 1). Note also that biomass estimates in the reserve under partial protection (all fishing gears banned except hook and line) (shaded data points in Fig. 2*b*)

Table 3. Results of repeated-measures ANOVA of fish biomass in reserve and nonreserve sites at Sumilon and Apo islands over the period 1983–2001.

Island	Source of variation	df	Mean square	*F* ratio	*p*
Sumilon	Reserve	1	0.042	0.08	ns
	Time	13	0.279	3.93	<0.001
	Reserve–time	13	0.235	3.30	<0.001
	Plots (reserve)	10	0.555	7.82	<0.001
	Residual	130	0.071		
	Total	167	1.182		
Apo	Reserve	1	11.124	40.42	<0.001
	Time	13	0.433	5.73	<0.001
	Reserve–time	13	0.344	4.56	<0.001
	Plots (reserve)	10	0.275	3.62	<0.001
	Residual	130	0.076		
	Total	167			

Note: Biomass estimates were made for five families of reef fish at Sumilon (Acanthuridae, Carangidae, Lutjanidae, Lethrinidae, and Caesionidae) and four families at Apo (same as Sumilon minus Caesionidae).
ns, $p > 0.05$.

were always less than those for full protection (solid data points in Fig. 2*b*). Biomass in the Sumilon nonreserve was generally stable during periods open to fishing (Fig. 3*b*; Table 1). Biomass in the nonreserve rose by 89.8% between 1985 and 1991 during 5 years of protection ((Fig. 3*b*; Table 1). Biomass declined by 70.5% between 1991 and 2001 when opened to fishing (Fig. 3*b*; Table 1).

The 42.7% decline in biomass of the five families of fish in the Sumilon reserve between 1983 and 1985 ($t_{5df} = 2.73$, $p < 0.05$) (Fig. 4*a*) coincided with a 40% decline in catch of traps and gill nets outside the reserve over the same period ($t_{2df} = 6.70$, $p < 0.01$) (Fig. 4*a*). The Sumilon reserve was then reprotected to varying degrees from fully protected (1987–1991) to all fishing gears but hook and line banned (1995–2001) (Fig. 3*a*; Table 1). The 27.2% increase in fish biomass inside the Sumilon reserve between 1985 and 2001 ($t_{5df} = 0.38$, $p > 0.05$) (Fig. 4*a*) coincided with a 26.9% increase in trap and gillnet catch outside the reserve over the same period ($t_{5df} = 0.38$, $p > 0.05$) (Fig. 4*a*). Total catch of targeted fish (minus Caesionidae) was significantly higher (by 52.7%) after (1985–2001) than before (1981) reserve establishment at Apo Island ($t_{6df} = 6.60$, $p < 0.01$) (Fig. 4*b*). A more conservative interpretation would be a comparison of the total catch at Apo before the reserve was established (1981) with that when the biomass in the reserve was very high (1998–2001). This comparison suggests a 41.3% increase in catch of these fish (Fig. 4*b*). The fish biomass increased significantly (by a factor of 4.6 over 18 years) inside the Apo reserve (linear regression, $F_{[1,12]} = 19.88$, $p < 0.001$) (Fig. 2*h*).

The relationship between biomass in the reserve and annual catch varied between the two islands (Fig. 2). At Sumilon, there were strong positive correlations between targeted fish biomass in the reserve ($F_{[1,11]} = 9.77$, $p < 0.01$) (Fig. 2*b*) and catch outside the reserve ($F_{[1,3]} = 4.93$, $p = 0.11$) (Fig. 2*c*) with years of reserve protection, although the latter relationship was not statistically significant owing to the low number of degrees of freedom of the test. At Apo, there was a strong positive relationship between targeted fish biomass in the reserve with years of reserve protection ($F_{[1,12]} = 19.88$, $p < 0.001$) (Fig. 2*h*) but no significant relationship between catch outside the reserve and years of reserve protection ($F_{[1,4]} = 0.21$, $p > 0.05$) (Fig. 2*i*).

Discussion

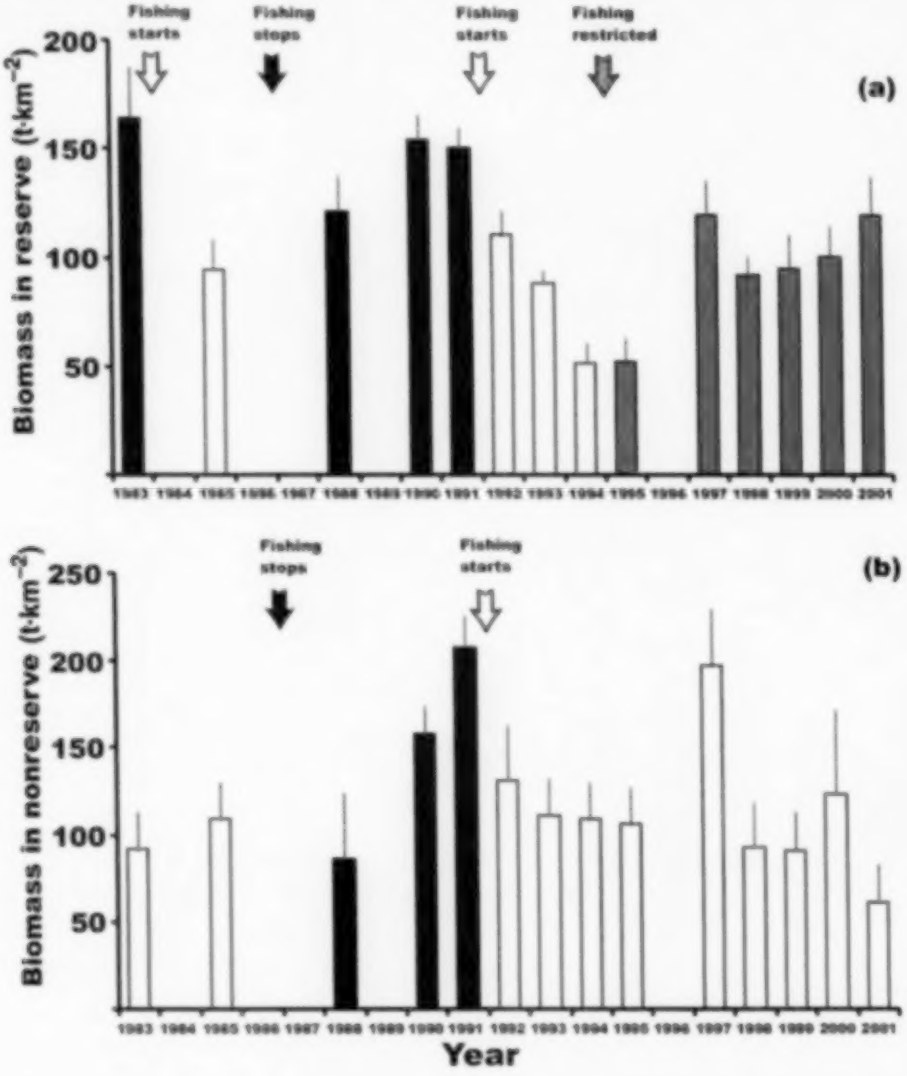
We have demonstrated that closure to fishing of 10%–25% of fishing area of two small islands in the Philippines did not reduce total fishery catch at the islands in the long term (two decades). On the contrary, the experimental evidence suggests that the total catch was sustained, or even enhanced, in the long term. These results are particularly significant, given that municipal (subsistence) fishing is such a major human activity at each island.

The prerequisite for beneficial fishery effects of no-take marine reserves beyond their boundaries is that biomass of targeted fish increases substantially over time inside relative to outside the reserves. Such patterns were clear at both Sumilon and Apo islands. Biomass was greater in reserve than fished areas after about 6 years of effective no-take protection at each island, and these differences became larger with increased duration of protection up to 19 years. The rates of biomass buildup inside the reserves were generally consistent with expectations based on life history characteristics of the fish (Russ and Alcala 1998). Large predators (Lutjanidae, Lethrinidae, and Carangidae) and many Acanthuridae (surgeonfish) are long-lived, often with low rates of natural mortality and recruitment. Such characteristics would suggest that recovery rates would be gradual over two decades, as observed. On the other hand, Caesionidae are generally short-lived, with high rates of natural mortality and recruitment (Russ and Alcala 1998).

In addition to long-term demonstrations of biomass buildup inside reserves, we have clear evidence, at least for Apo Island, of mechanisms by which the no-take reserve may have influenced the local fishery (Russ and Alcala 1996; Russ et al. 2003, 2004). These mechanisms are spillover from the reserve (Russ and Alcala 1996; Russ et al. 2003) and a general improvement in the reef resources associated with the implementation of a marine management plan in 1986, which included establishment of the no-take reserve (Russ et al. 2004).

This study expands and combines our previous studies of these two reserves and their adjacent fisheries. For the first time, we show the dynamic response of biomass of targeted fish at the Sumilon reserve and nonreserve to changes in fishing levels over the period 1983–2001. The observations that both reserve biomass and catch at Sumilon were initially high after almost a decade of protection (1983), declined by 1985–1986 after intense fishing, and then recovered again by 2001 are novel. At Apo, both initial biomass and prereserve catch were low at the beginning of the study. Reserve biomass increased substantially over almost two decades of protection, and catch was higher or at least remained relatively stable. Combined, these studies constitute a unique, spatially replicated natural experiment.

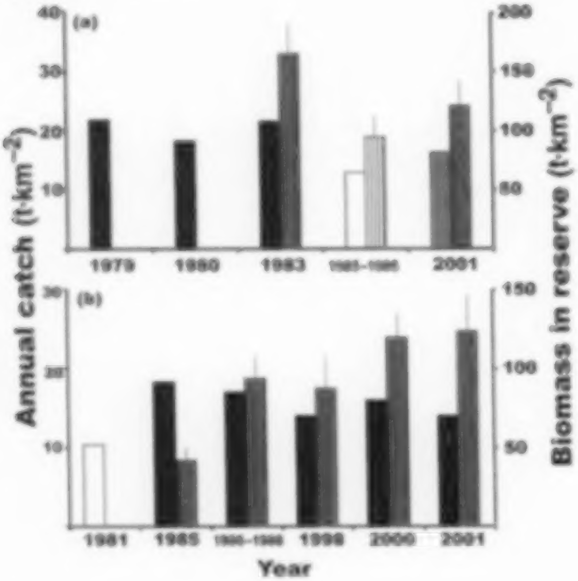
Fig. 3. Underwater visual census estimates of biomass of five families of reef fish (Acanthuridae, Carangidae, Lutjanidae, Lethrinidae, and Caesionidae) in (*a*) reserve and (*b*) nonreserve sites at Sumilon Island from 1983 to 2001. Arrows show the change in fishing status over time. Error bars are SEs.



There are several alternative explanations for our results, other than biomass buildup in the reserves influencing the adjacent fishery. A general improvement in environmental conditions could lead to more biomass in the reserves and concomitantly more catch outside them. However, at Apo, we have shown that biomass of some of these targeted fish increased over time outside the reserve close to (within 200 m) but not far from the reserve (Russ and Alcala 1996; Russ et al. 2003, 2004). Such a pattern is consistent with spillover rather than a general improvement of the entire reef. Increased biomass in a reserve associated with greater catch outside it could also occur if the fishing effort expands in response to reserve establishment. Overall fishing effort (numbers of fishers and numbers of traps) remained very stable at Sumilon for the study. At Apo, fishing effort of the main fishing gear (hook and line) declined over the study period (Russ et al. 2004). We do concede that the implementation of the marine management plan at Apo in 1986, a plan that banned all forms of destructive fishing gears and fishing by nonresidents, confounds any effects of the reserve alone on the local fishery. This is likely to have led to a general improvement in the reef resources and thus potential catch.

The results presented here are subject to a number of other potential limitations. One limitation was that we restricted the study to just five families of fish at Sumilon and four at Apo. We did this for two reasons. Firstly, these fami-

Fig. 4. Total annual catch from the nonreserve areas (open columns) and underwater visual census estimates of biomass in the no-take reserves (columns with vertical lines) at (*a*) Sumilon Island (1979–2001) and (*b*) Apo Island (1981–2001). Note the differences in vertical scales for catch and reserve biomass. At Sumilon, the annual catch and reserve biomass is for five families that constitute 92% of the catch at the island (Acanthuridae, Carangidae, Lutjanidae, Lethrinidae, and Caesionidae). Catch at Sumilon is trap and gillnet catch, gears that take 64% of the catch at the island. At Apo, the annual catch and reserve biomass is for four families that constitute 76% of catch at the island (same as for Sumilon minus Caesionidae). At Apo, the catch is hook and line, trap, gill net, and spear catch, gears that take 100% of the catch at the island. Reserve status was no protection (open columns), full no-take protection (solid columns), or partial protection (all gears but line fishing banned) (shaded columns).



lies constitute the majority of the yield, 92.3% and 75.6% by weight, at Sumilon and Apo, respectively. Secondly, they were amenable to underwater visual census, and thus relatively accurate monitoring of abundance inside and outside the reserves was possible. One of these five families was omitted from the Apo analysis (Caesionidae). This family made up only a small proportion of the yield at this island (Maypa et al. 2002). It also displayed a distinct reduction in abundance inside the Apo reserve from 1992 onwards, possibly associated with reduced recruitment, independent of reserve effects (G.R. Russ, unpublished data). We also restricted ourselves to just two fishing gears at Sumilon (traps and gill nets). These were the only two gears for which we had relatively long-term data (1979–2001). They accounted for approximately 64% of the yield at Sumilon Island. At Apo Island, we had long-term catch data for all of the four main gears (hook and line, traps, gill nets, and spear).

Another limitation of our study was that critical information was often based on a single replicate. Thus, we had only one measurement of reserve biomass and yield at Sumilon after the reserve was fished down in 1985 and only one measurement of these variates after protection was reapplied (2001). At Apo Island, we had only one measurement of yield before reserve establishment. Finally, slight variations in methods used to collect catch data over the period 1983–2001 may have influenced comparisons of catch over time at each island. Generally, most studies at each island used similar methods and sampled over most of any particular year. However, the one measurement of yield made before reserve establishment at Apo (Alcala and Luchavez 1981) was based on just 49 days of sampling and is thus a less reliable estimate. Such limitations were largely unavoidable.

On the other hand, the study has some unique advantages over the few other studies of the effects of no-take reserves on total fishery yields. Both experiments at Sumilon and Apo have aspects of a before-after-control-impact-pairs experimental design, with before and after data taken at a reserve and fished site at each of two islands. The Sumilon study even incorporated experimental removal and reapplication of reserve status, somewhat analogous to a crossover experimental design encouraged in fisheries experiments (Hilborn and Walters 1992). Our study is also relatively unique in having long-term (18 years) monitoring of fish biomass inside and outside well-protected no-take reserves at two islands coupled with five (Sumilon) and six (Apo) independent measurements of total fishery yield taken over two decades at each island. Clearly, the study takes advantage of two natural experiments that arose from the actions of local fishing communities as opposed to properly designed scientific experiments. Furthermore, while the study is experimental, it relies on correlations between duration of reserve protection and either reserve biomass or local catch. It does not explicitly establish mechanisms by which biomass in reserves may have influenced local catch.

However, in other studies, we have documented one potential mechanism, spillover, to partly explain the neutral to positive results of reserve biomass on catch reported here. At Apo Island, we demonstrated gradual buildup of biomass just outside the southern boundary of the reserve of large predatory fish (Russ and Alcala 1996), Carangidae (jacks) and Acanthuridae (surgeonfish) (Russ et al. 2004), and the large surgeonfish *Naso vlamingii* (Russ et al. 2003). We have also demonstrated higher hook and line CPUE of Acanthuridae and *N. vlamingii* closer to (within 200 m) than farther from the Apo reserve boundary in 2000–2001 (Russ et al. 2003, 2004). These studies are all consistent with the hypothesis of spillover from the Apo reserve affecting the local fishery in a positive manner. At Sumilon Island, a model incorporating movement rates across the reserve boundary suggested that spillover from the reserve could have increased yield per recruit (and thus total yield) of fusiliers (Caesionidae), the major component of the catch (Russ et al. 1992).

Few other studies have addressed the key question of effects of no-take reserves on total fishery yields. Alcala and Russ (1990) reported a significant reduction in total catch following the breakdown of protection of the Sumilon reserve in the Philippines, a study that forms part of the present research. Russ et al. (2004) showed that the benefits of the reserve to local fisheries for surgeonfish and jacks at

Apo Island over a 20-year period were higher or sustained catch, increased hook and line CPUE, and a reduction in fishing effort. The fishery and tourism benefits generated by the Apo reserve have enhanced the living standards of the fishing community (Russ et al. 2004).

McClanahan and Kaunda-Arara (1996) and McClanahan and Mangi (2000) reported increased CPUE near the boundary of the Mombasa no-take marine park but a 35% reduction in total catch when the park removed 65% of the local fishing area. Thus, the no-take park had a negative effect on total yield but not as much as expected if the reserve simply reduced catch in direct proportion to the fishing area removed. Some positive effects on the fishery, possibly by spillover, likely occurred. In St. Lucia, Roberts et al. (2001) showed that a network of no-take reserves that closed 35% of local fishing grounds resulted in 46%–90% increases in CPUE after 5 years of reserve protection. These reserves thus had a positive effect on total fishery yield. The Philippine, Kenyan, and St. Lucia studies all measured fish biomass inside and outside reserves, plus adjacent fishery yields, both before and after reserve establishment. Closure to fishing of three large areas of Georges Bank in 1994 increased abundance of scallops (*Placopecten magellanicus*) substantially (Murawski et al. 2000). Total catch of the scallop fishery increased between 1994 and 1998, despite the reduced fishing area. Fishing effort concentrated outside the boundaries of the closed areas, particularly in places most likely to receive scallop larvae exported from the closed areas (Gell and Roberts 2002). These results suggest that the no-take reserves had a positive effect on total yield of scallops on Georges Bank. Other very good examples of potential spillover from no-take reserves that did not address the question of the reserve effect on total yield directly include those of Kelly et al. (2002) on lobsters in New Zealand and Galal et al. (2002) on reef fish in the Egyptian Red Sea.

The mechanisms by which the reserves in the examples above may have affected fishery yield probably differ. The relatively small spatial scale of the studies in the Philippines, Kenya, and St. Lucia led all of the authors to suggest that spillover of adults was the most likely mechanism affecting yields beyond the boundaries of the reserves. These effects included increased local yields (Sumilon and St. Lucia), a somewhat neutral effect on yield (Apo), or decreased yield but not decreased as much as expected based on the amount of fishing area removed (Mombasa). The increased total yield of scallops on Georges Bank following establishment of no-take areas is most likely due to the recruitment effect. Until recently, it was assumed that any positive influences of reserves on local CPUE and yields were due to adult spillover. Recent studies of the potential for limited larval dispersal and "self-recruitment" (e.g., Jones et al. 1999; Swearer et al. 1999; James et al. 2002) have led to the possibility that reserves may affect local fishery yields by both spillover and the recruitment effect (Gell and Roberts 2002).

In conclusion, our study has demonstrated that closure of 10%–25% of available fishing area of two small islands in the Philippines resulted in sustained, or even enhanced, total catch of the local fishery in the long term. The result challenges the frequent suggestion that no-take reserves will result in reduced total catch. The latter may occur in the short term. But if targeted fish biomass builds up substantially inside no-take reserves, the potential for them to sustain, or even enhance, fishery yields by spillover or enhanced recruitment into fished areas is very real.

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Assessing the potential for stock enhancement in the case of the Chesapeake Bay blue crab (*Callinectes sapidus*)

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Abstract: In certain cases of severely depleted fishery stocks, combining stock enhancement with traditional management techniques may be a useful way of returning stocks to an exploitable size. The Chesapeake Bay stock of blue crabs (*Callinectes sapidus*) has declined over the past decade and appears to be recruitment-limited, making it an appropriate candidate for enhancement efforts. This study serves as a first step in determining whether large-scale enhancement of blue crab stocks is feasible. Four hatchery-raised cohorts of 4000 – 10 000 (25 000 in total) juvenile (6–30 mm carapace width, 58–70 days old) crabs were released in upper Chesapeake Bay coves. Sixty days after release, these crabs constituted 22%–79% of all crabs in the hatchery-crab size range (corresponding to an enhancement level of 28%–366%). Crabs released earlier in the summer reached maturity at the age of 6 months, younger than their wild counterparts. Estimated survivorship to maturity was 16%–20% for early-released crabs and 5–15% for late-released crabs. Late-released crabs, like wild crabs, had to overwinter before becoming mature. Our study suggests ways to improve success of hatchery-raised individuals that can be broadly applied across taxa. The results also contribute specifically to determining whether large-scale stock enhancement is possible in the case of the Chesapeake Bay blue crab.

Résumé : Chez certains stocks fortement réduits, la combinaison de mesures d'amélioration des stocks et de méthodes de gestion courantes peut être utile pour rétablir les stock à une taille exploitable. Le stock de crabes bleus (*Callinectes sapidus*) de la baie de Chesapeake a décliné au cours de la dernière décennie et semble limité par des problèmes de recrutement; c'est donc un bon candidat pour les efforts d'amélioration des stocks. Notre étude constitue une première étape pour déterminer si une amélioration du stock de *C. sapidus* à grande échelle est possible. Quatre cohortes de 4 000 à 10 000 (total de 25 000) jeunes (largeur de carapace, 6–30 mm; âge, 58–70 jours) crabes de culture ont été relâchés dans les anses supérieures de la baie de Chesapeake. Soixante jours après l'ensemencement, ces crabes représentaient 22–79 % des tous les crabes des classes de taille des crabes de culture, ce qui correspond à un niveau d'amélioration de 28–366 %. Les crabes relâchés plus tôt au printemps atteignent la maturité à l'âge de 6 mois, donc plus rapidement que les crabes sauvages. La survie à la maturité est estimée à 16–20 % chez les crabes relâchés tôt et à 5–15 % chez les crabes relâchés tard. Les crabes relâchés tard, tout comme les crabes sauvages, doivent passer l'hiver avant d'atteindre la maturité. Notre étude suggère des méthodes pour augmenter le succès d'individus élevés en culture qui sont largement applicables à d'autres taxons. Nos résultats contribuent aussi de façon spécifique à évaluer si des améliorations de stock à grande échelle sont possibles chez le crabe bleu de la baie de Chesapeake.

[Traduit par la Rédaction]

Introduction

Stock enhancement has been used in the management of severely exploited fisheries for over a century in several areas around the world with various degrees of success. Enhancement efforts encompass many approaches, such as increasing the amount of habitat to bolster habitat-limited populations and releasing juveniles to augment recruitment-limited populations (e.g., Masuda and Tsukamoto 1998; Castro et al. 2001). Within the approach of releasing juveniles, the range of techniques includes seeding areas in cases of sessile species (e.g., Barbeau et al. 1996), releasing mobile juveniles into isolated areas where they can be followed (e.g., Davenport et al. 1999), and releasing juveniles into the open ocean (Masuda and Tsukamoto 1998).

In an ideal stock-enhancement program directed toward recruitment-limited populations, individuals are reared in an aquaculture facility until they are beyond the initial phase of high early-life-history mortality and are then released into the natural population. Ideally, after release, these hatchery-

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Table 1. Aspects of the four batches (A–D) of hatchery-raised blue crabs (*Callinectes sapidus*).

Batch	Hatch date	Release date	Batch size (no. of crabs)	Release site (cove)[a]	Cove size (ha)	Mouth length (m)[b]	Initial density (no.·m^{-2})[c]
A	14 Feb.	2 May	3800	Boathouse Creek, RR	5.5	120	0.07
B	13 May	19 July	4800	Boathouse Creek, RR	5.5	120	0.09
C	17 June	15 Aug.	9600	Logan Pond, SR	3.0	5	0.33
D	30 June	9 Sept.	7000	Sheepshead Cove, RR	3.5	15	0.20

[a] RR, Rhode River; SR, South River.
[b] Size of the cove opening.
[c] Based on the number of hatchery-raised crabs initially released.

reared individuals contribute to the spawning stock, with a potential exponential impact on overall population size in subsequent generations depending on the degree of recruitment limitation.

Because stock enhancement may be accompanied by potential problems, enhancement is a controversial management method for many reasons (Washington and Koziol 1993; Bell and Gervis 1999; Li 1999). Hatchery-raised animals may not survive in the wild. Release of genetically homogeneous hatchery-raised individuals may reduce genetic variability in wild populations through inbreeding (Tringali and Bert 1998; Utter 1998). Hatchery animals may compete with and displace wild animals (Bannister et al. 1994; Castro et al. 2001). The potential for enhancement success may reduce or excuse management activities needed to reduce fishing effort or habitat degradation (Lichatowich 1999). Finally, increases in stock size due to hatchery successes may provoke a rise in fishing effort, and therefore greater pressure on the remaining wild individuals (Hilborn 1998; Bannister 2000). The controversy is fueled by the fact that most enhancement efforts, which date from at least a century ago, have not been studied in a quantitative manner (Bannister et al. 1994; Heppell and Crowder 1998; Crowe et al. 2002). Such study did not begin until the late 1980s (Leber 1999).

The recent recognition of these problems has led in some cases to refinement of the enhancement process. Methods to better select candidate species have been developed (Blackenship and Leber 1995). Advances in tools such as tagging have allowed better assessment of survivorship of both hatchery-raised and wild animals (Blackenship and Leber 1995). Most importantly, calls have been made for quantitative study of small-scale enhancement efforts before investment in large-scale programs begins (Leber 1999; Crowe et al. 2002). To date, such studies have concentrated on finfishes, as this group is the focus of most enhancement efforts (Secor and Houde 1998; Thorpe 1998; Wilson et al. 1998). For example, Japan has enhancement programs for 34 finfishes but only 12 crustaceans (Masuda and Tsukamoto 1998). Mollusks (but see Kojima 1995; Arnold 2001; Beal et al. 2002) and crustaceans (but see Bannister and Addison 1998; Su 1988; Castro et al. 2001), groups with different life cycles and habitats and for which different fishing methods are used, have received less attention.

The goal of the present study is to provide a quantitative assessment of small-scale experimental enhancement of the blue crab, *Callinectes sapidus*. This crab, ecologically important, also supports the most economically important fishery in Chesapeake Bay, Maryland, USA (Rugulo et al. 1998). Over the past decade, spawning-stock abundance has declined by 81%, mean size at maturity by 9%, and postlarval recruitment by an order of magnitude (Lipcius and Stockhausen 2002). The optimal means of achieving sustainable stocks of highly exploited species such as the blue crab is to manage the natural population effectively (Hilborn and Walters 1992). However, the level of decline exhibited by the Chesapeake Bay population suggests that effective management may ultimately require, in addition to more traditional management techniques, responsible stock enhancement aimed at restoring breeding stocks.

Though the success of some enhancement efforts has begun to be quantified for several decapods, mostly lobsters (e.g., Bannister and Addison 1998; Castro et al. 2001), these species have very different life histories than blue crabs in the Chesapeake Bay. The most similar decapod for which stock enhancement has been attempted is the swimming crab, *Portunus trituberculatus*, of which 28–42 million hatchery-reared juveniles are released annually in Japan (Masuda and Tsukamoto 1998; Ariyama 2000; Secor et al. 2002). However, analysis of enhancement success of the *P. trituberculatus* program has been minimal (Secor et al. 2002). In this study, we aimed to determine whether enhancement of blue crab populations is possible at small spatial scales. Specific goals were to (*i*) determine survivorship of hatchery-raised crabs released into the field, (*ii*) measure how long hatchery crabs remain in local release areas, (*iii*) compare growth rates of crabs released at various times of the spring and summer, and (*iv*) determine whether hatchery crabs displace wild crabs or populations are far enough below carrying capacity that overall population size is enhanced by the release of hatchery crabs. Our goal was to provide a rigorous, quantitative test of the potential for stock enhancement on an initially small scale for blue crabs in Chesapeake Bay.

Methods

Hatchery-raised crabs

Hatchery-raised blue crabs were produced at the University of Maryland Biotechnology Institute's Center of Marine Biotechnology (UMBI-COMB) according to methods described by Zmora et al. (2005). Four batches of crabs were hatched in February (batch A), May (batch B), mid-June (batch C), and late June 2002 (batch D) from four mature females collected from Chesapeake Bay and therefore already storing the sperm of wild males. Approximately 2 months after hatching and 1 month after metamorphosis to first instar, at a mean size of 18 mm carapace width (CW) (range 6–30 mm CW), crabs in each batch were tagged with visual-implant elastomer, microwire, or both (Davis et al. 2004*a*).

Fig. 1. Locations of several of the sites used in Chesapeake Bay, USA. Sheepshead Cove and Boathouse Creek were enhancement sites and Cheston Creek was used as a control site. The arrow in *a* shows the location of Chesapeake Bay within the USA and the arrow in *b* indicates the location of the study sites within Chesapeake Bay.

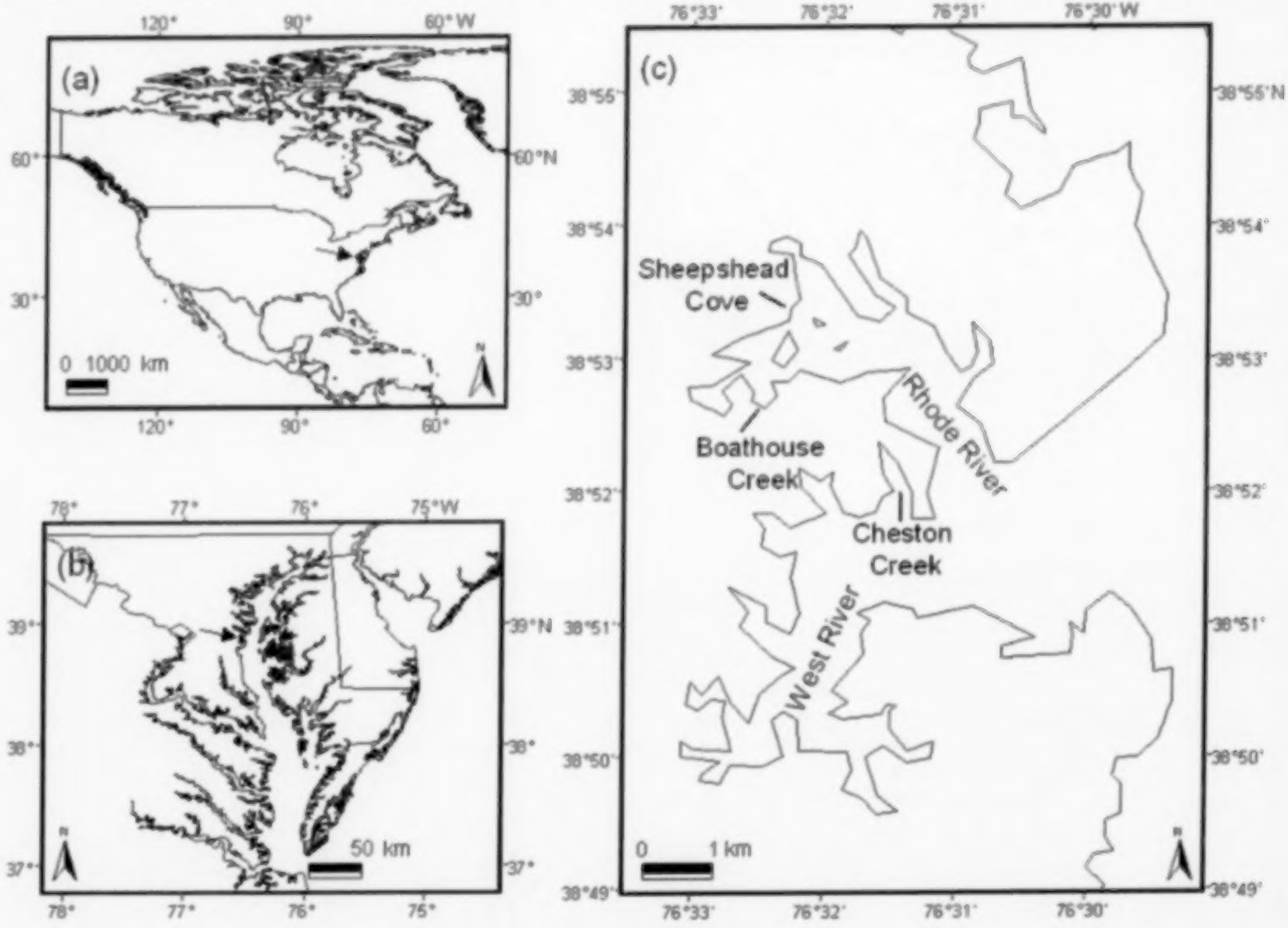
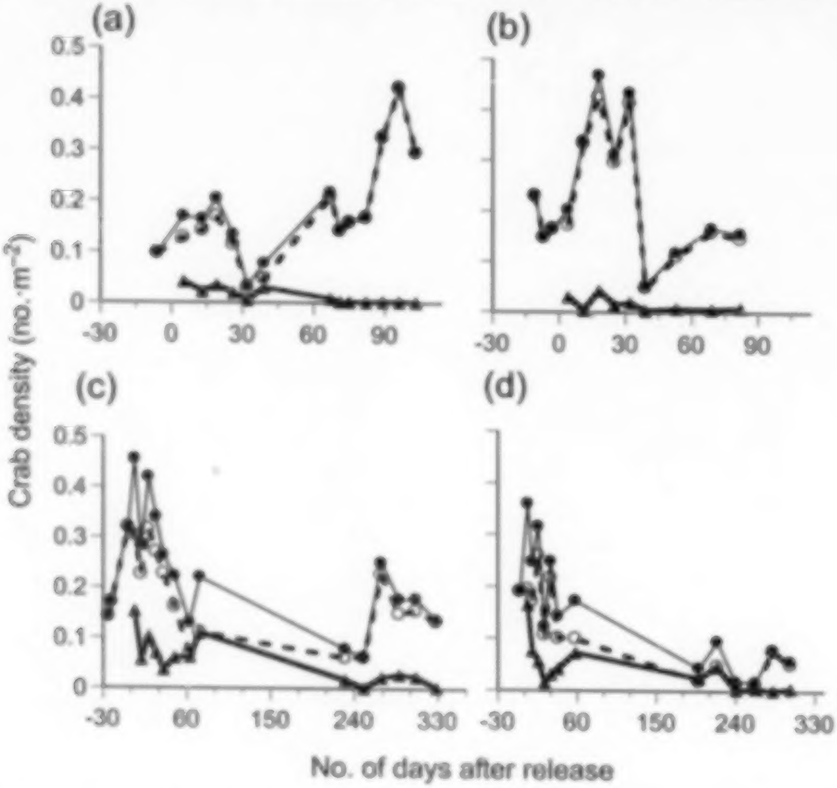

(a)
120°
90°
60° W
60°
60°N
30°
30°
0 1000 km
N
(b)
78°
77°
76°
75°W
39°
39°N
38°
37°
50 km
(c)
76°33'
76°32'
76°31'
76°30' W
38°55'
38°55'N
38°54'
38°53'
38°52'
38°51'
38°50'
38°49'
Sheepshead Cove
Boathouse Creek
Rhode River
Cheston Creek
West River
0 1 km

Fig. 2. Densities of blue crabs (*Callinectes sapidus*) in the enhancement sites (- -○- -, all wild crabs; —▲—, all hatchery-raised crabs; —●—, total crabs (hatchery + wild)). (*a*) Batch A, which had an average enhancement value of 7%. (*b*) Batch B, also with an enhancement value of 7%. (*c*) Batch C, which had an enhancement level of 39%. (*d*) Batch D, with an enhancement level of 40%.



The number of released crabs in each batch ranged from 3800 to 9600 (Table 1).

Enhancement and control sites

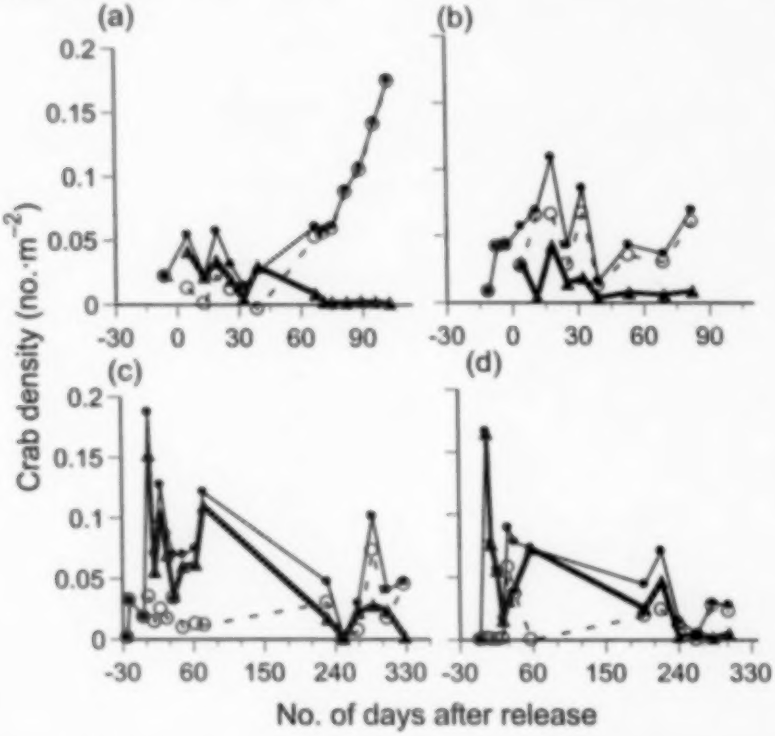
The batches of tagged hatchery crabs were released into the wild in three semi-enclosed coves (Boathouse Creek, Sheepshead Cove, and Logan Pond) in the Rhode and South rivers, two subestuaries of the upper western Chesapeake Bay (Table 1; Fig. 1). Semi-enclosed coves were chosen to encourage retention of crabs within the sampling region, thereby limiting the loss of hatchery crabs due to emigration that would be erroneously attributed to mortality. Crabs were distributed along several 50- to 100-m lengths of shoreline near the heads of the coves, away from the cove mouths. These regions are referred to as release areas. The ambit scale of crabs in this small size range has not been studied in detail, but previous studies of juvenile crabs in similar areas (van Montfrans et al. 1991) and in these coves (Davis et al. 2004*b*) indicated that few emigrated over a period of several weeks.

Wild crab densities were also measured in several control areas both before and after release of hatchery crabs of batches B, C, and D in the experimental sites. These dates of control-site sampling fell within 1 week, usually within 1 or 2 days, of experimental-site sampling. Several different control sites were used for comparisons. Cheston Creek in the West River (Fig. 1) was used as a control site for the releases of batches B and C. Sheepshead Cove, prior to the release of batch D, was used as a control site for batch C. Logan Pond, prior to the release of batch C, was used as a control site for batch B. Boathouse Creek, though hatchery crabs had been released here 1 month before batch C and 2 months before batch D, was used as an additional control site for these last two batches, ignoring those hatchery crabs present. The main stem of the Rhode River was used as a control site for batches B, C, and D.

Field sampling

Each cove was sampled 1–3 times before the addition of hatchery crabs to determine background levels of wild blue crabs, and at approximately weekly intervals after release of each cohort. Two sampling methods were used: beach seining and epibenthic sled tows. Beach seines were 16 m long, were 3 m tall, had 6 mm mesh, and were pulled 25 m parallel to shore by two people standing approximately 9 m apart, and therefore sampled approximately 225 m^2 in surface area. The 1 m wide, 50 cm tall epibenthic sled had a 2 m long, 6 mm mesh net with a 500-μm cod-end. The sled was pulled for 100 m by a shallow-draft boat powered by a small outboard motor. Gear efficiency was determined in separate studies: for the seine net by releasing known numbers of tagged crabs of 10–130 mm CW into enclosed regions and

Fig. 3. Densities of just wild crabs in the hatchery crab size range (- -○- -), all hatchery crabs (—▲—), and total crabs (hatchery + wild) in the hatchery crab size range (—●—) in the enhancement sites. (*a*) Batch A, which had an average enhancement value of 24%. (*b*) Batch B, with an enhancement value of 35%. (*c*) Batch C, which had an enhancement level of 225%. (*d*) Batch D, with an enhancement level of 352%.



seining immediately afterward (J.L.D. Davis and A.C. Young-Williams, unpublished data), and for the sled by towing over known densities of tagged crabs (A.C. Young-Williams and J.L.D. Davis, unpublished data).

Both pre- and post-release sampling was conducted at predetermined sites within each cove. Owing to the large area surrounding the coves and the low probability of recovering tagged crabs outside the focus regions, sampling was confined to the coves. In Boathouse Creek, 10 seining stations were distributed around the cove perimeter and 21 sled-tow sites were identified throughout the cove. Logan Pond contained 6 seine and 10 sled-tow sites. Sheepshead Cove had 5 seine and 10 sled-tow sites. All major habitat zones of each cove were represented; however, sampling sites were not distributed uniformly. Sampling effort was proportionally high in crab release areas. In total, 7% of the surface area of each cove was sampled; however, 25%–32% of the area within 300 m of the initial release points was sampled, as opposed to 4%–5% of non-release areas. Consequently, calculations of population abundance were adjusted to account for the initial distribution of sampling efforts.

For all blue crabs collected in each seine and sled tow, both tagged and wild, we measured CW and identified gender. All crabs were then returned to the cove in the approximate catch location. Sampling was conducted in each cove usually once per week, though at times once per 2 weeks, until tagged crabs were no longer recaptured (4–12 months). However, sampling was discontinued during December–February, when crabs cease activity.

All control sites but the Rhode River main stem were sampled with the same gear (beach seines and epibenthic sled) as the release sites. In the Rhode River main stem, crab densities were sampled in four 2250-m^2 areas with a 2.5-m otter trawl (as described in Hines et al. 1987) at times matching "before" and "after" sampling of the three batches B, C, and D. Though the sampling equipment was different, before and after comparisons of crab densities at this site provided an indication of whether background crab densities changed.

Data analysis

The number of hatchery crabs in the release and non-release areas of each cove during each sampling period was estimated as the number of tagged crabs collected plus the number of crabs estimated to have lost their tags (see Davis et al. 2004*a*) and corrections for catch efficiency, divided by the percentage of each area (release or non-release) sampled. To account for the difference in sampling proportions for release areas versus non-release areas, crab abundances were estimated separately for these two area types.

The number of hatchery crabs (H) present in an embayment at time t was therefore estimated as

$$H = (N_R / A_R + N_{NR} / A_{NR}) \tag{1}$$

Fig. 4. Contribution and enhancement of hatchery crabs. Contribution is defined as the proportion of all crabs that are of hatchery origin (hatchery/total crab densities). Enhancement is defined as the increase over wild crabs provided by hatchery crabs (hatchery/wild densities). (*a* and *c*) Contribution and enhancement of hatchery crabs, respectively, considering all wild crabs. (*b* and *d*) Contribution and enhancement of hatchery crabs, respectively, considering only wild crabs in the hatchery crab size range (—●—, batch A; —■—, batch B; - -○- -, batch C; - -□- -, batch D).

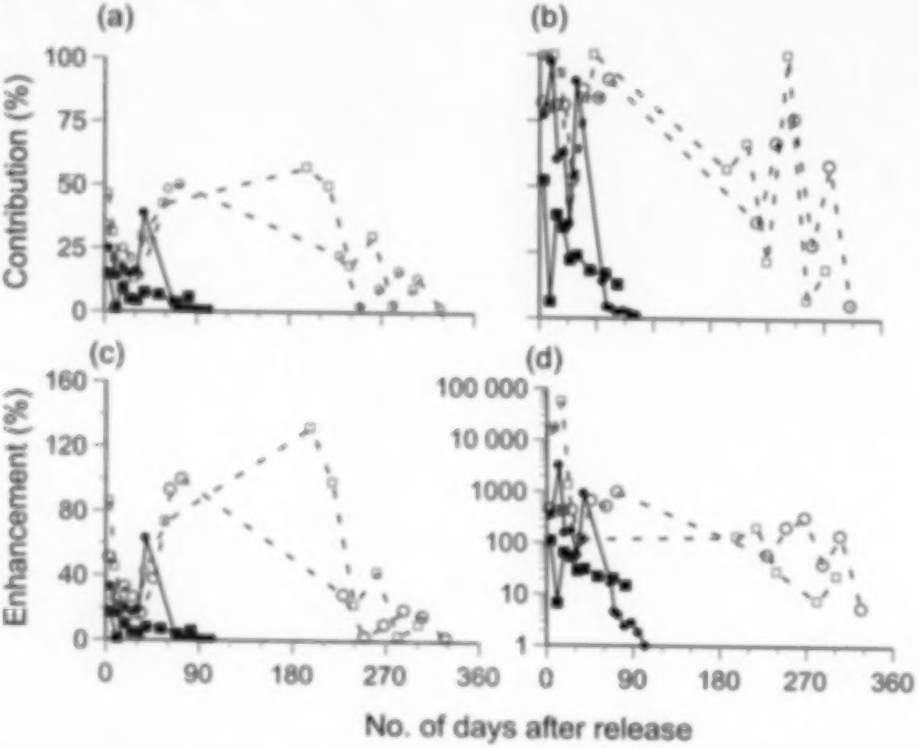
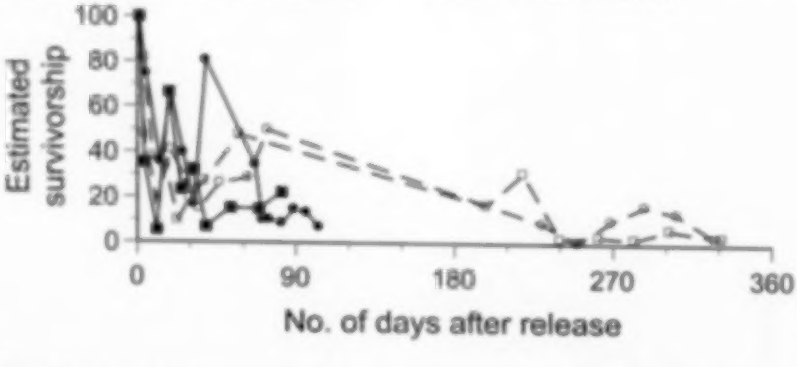


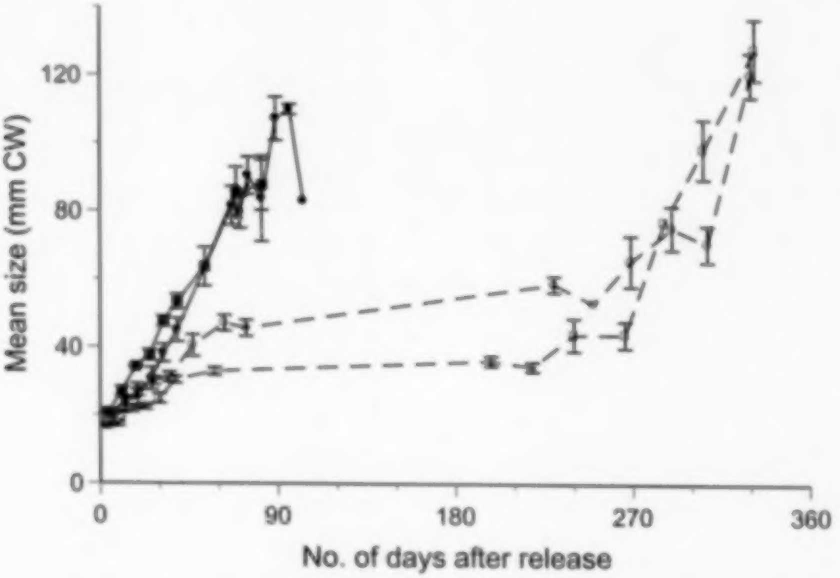
Fig. 5. Estimated survivorship of each hatchery batch over time after release into the field. Survivorship values are calculated as the percentage surviving from the initial release date to the time indicated on the *x* axis. The *p* value listed represents significance of the test for differences in slope (—●—, batch A; —■—, batch B; - -○- -, batch C; - -□- -, batch D).



where N_R is the number of hatchery crabs in the release area; A_R is the percentage of the release area that was sampled (between 19% and 26% for the four coves); N_{NR} is the number of tagged crabs in the non-release area; and A_{NR} is the percentage of the non-release area that was sampled (between 4% and 5%). The estimated number of tagged hatchery crabs (N_{tagged}) present in each area (release or non-release) at time *t* incorporates gear-specific estimates of gear efficiency (GE):

$$N_{tagged} = (N_{tow}/GE_{tow} + (N_{small\ seine}/GE_{small\ seine}) + N_{large\ seine}/GE_{large\ seine}) \tag{2}$$

Fig. 6. Mean size (carapace width, CW) of hatchery crabs over time after their release into the field. Blue crabs are legally harvestable as soft crabs at 100 mm CW (soft-shell fishery) and 125 mm CW (hard-shell fishery). They reach maturity at approximately 120 mm CW. Error bars represent standard errors. The number of crabs contributing to each mean ranged from 10 to 158 within the first 30 days after release and from 1 to 36 beyond 30 days after release (—●—, batch A; —■—, batch B; - -○- -, batch C; - -□- -, batch D).



where N_{tow} is the number of tagged crabs recaptured in the tows; $N_{\mathrm{small\ seine}}$ and $N_{\mathrm{large\ seine}}$ are the numbers of small (<20 mm CW) and large (≥20 mm CW) tagged crabs recaptured with seines, respectively; $\mathrm{GE}_{\mathrm{tow}}$ is the tow-gear efficiency (5.5%); and $\mathrm{GE}_{\mathrm{small\ seine}}$ and $\mathrm{GE}_{\mathrm{large\ seine}}$ are the gear efficiency for small (10%) and large (50%) crabs, respectively. Estimates of the total number of hatchery crabs (N) present in an area (release or non-release) requires a correction for tag loss, which is related to the mean size of hatchery crabs at time t (Davis et al. 2004*a*):

$$(3)\qquad N = N_{\mathrm{M}}/[1-(0.63\mathrm{CW}-11.1)] + N_{\mathrm{E}}/(1-0.77e^{0.066\mathrm{CW}})$$

where N_{M} is the number of hatchery crabs tagged with microwire; CW is the mean size of crabs caught at time t; and N_{E} is the number of hatchery crabs tagged with elastomer.

Survivorship (S) of crabs in each batch at a given time t was then calculated as

$$(4)\qquad S_t = (H_t/E_t)/H_0$$

where H_t is the estimated number of hatchery crabs present in a cove at time t; E_t is an emigration coefficient; and H_0 is the initial number of hatchery crabs released into the embayment. E, estimated from a telemetry experiment described in Davis et al. (2004*b*), was time-dependent and estimated as a step function: for the first 2 weeks $E = 1$, after which $E = 0.833$. Most likely, more than 16.7% of the hatchery crabs emigrated from the embayment over time (e.g., see Hines et al. 1995); however, with no data to support this hypothesis (because juvenile crabs molt often, telemetry can only be used for the duration of one molting cycle), we used this conservative estimate.

Two corrections applied to densities of hatchery crabs were also applied to estimates of densities of wild crabs. In the event that wild crabs were also not distributed evenly within the release coves, sampling area (release or non-release) was also taken into account when calculating the density of wild crabs. Corrections were also made for gear efficiency, though not for emigration or tag loss.

A number of ways exist to consider enhancement, and we used several in order to maximize comparability with other studies. First, we calculated the percentage of all crabs at a site that were hatchery-raised, which we refer to as the contribution:

$$(5)\qquad \mathrm{Contribution} = N_{\mathrm{H}}/(N_{\mathrm{H}} + N_{\mathrm{W}}) \times 100$$

where N_{H} is the number of hatchery crabs in an embayment and N_{W} is the number of wild crabs. Second, we calculated "enhancement" as

$$(6)\qquad \mathrm{Enhancement} = N_{\mathrm{H}}/N_{\mathrm{W}} \times 100$$

which can be reorganized into

$$(7)\qquad \mathrm{Enhancement} = \mathrm{contribution}/(100 - \mathrm{contribution}) \times 100$$

Hatchery contribution and enhancement, in turn, were considered (*i*) relative to the entire wild population and (*ii*) in terms of only the segment of the population (those crabs within the size range of our hatchery crabs (±10% of the extreme sizes)) that was enhanced.

Levels of contribution were compared among the four batches with a repeated-measures analysis of variance

(ANOVA) using weekly values at seven time periods: weeks 1, 2, 3, 4, 5, 6–7, and 8–9. Contribution values were arcsine square root transformed to meet assumptions of normality. To obtain values of hatchery-crab density and contribution at specific time points (30 and 60 days after release) for qualitative comparisons of the four batches, logarithmic regression equations were fit to relationships of these factors with time. Estimated values of density and contribution were calculated based on these equations.

To determine whether survivorship differed among the four batches within the first 3 months of release, we used analysis of covariance (ANCOVA) in which batch was a categorical factor and time (number of days after release) was a contiguous covariate. To meet assumptions of normality, arcsine square root transformations were applied to survivorship values and number of days after release was log-transformed. Crab growth rate was similarly compared with mean size of hatchery crabs on a sampling day as a response variable, batch as a factor, and time (number of days after release) as a covariate.

Assessing wild crab displacement

Two methods were used to assess whether the release of hatchery crabs resulted in displacement of wild crabs in the release coves. First, all coves were sampled 1–2 weeks both before and after hatchery releases. To determine whether an immediate decline in the wild population occurred, two-way fixed effects ANOVAs were used to compare wild crab densities between the "before" time period and 1 week after release, and between "before" and 2 weeks after release. In these ANOVAs, crab batch (A, B, C, and D) and time period (before and after) were factors. Second, we used *t* tests to compare the change in wild crab density from "before" to "after" time periods between control and experimental coves for batches B, C, and D.

Results

Enhancement, contribution, and survivorship

Densities of hatchery crabs, which were released at levels of 0.07–0.33·m^{-2} (Table 1), were estimated after 60 days in the field to range from 0.008 to 0.06·m^{-2} (Fig. 2). Densities of hatchery crabs were always less than total densities of wild crabs (Fig. 2) but often, especially for batches C and D, greater than densities of wild crabs in the size range of hatchery crabs (Fig. 3). Crabs in batches A and B, released early in the summer, experienced losses (death and emigration) during the midsummer period, when wild juveniles were moving up Chesapeake Bay and settling into upper-Bay habitats, causing an increase in the wild population (Figs. 2*a*, 2*b*, 3*a*, 3*b*). In contrast, crabs in batches C and D were released after the temporal peak in wild population size, and therefore changes in densities of hatchery and wild crabs over time were more similar (Figs. 2*c*, 2*d*, 3*c*, 3*d*).

Considering only wild crabs within the size range of hatchery-raised individuals (target population segment), the contribution of hatchery crabs in the four batches ranged from 32% to 85% 30 days after release and from 22% to 79% 60 days after release (Fig. 4). Corresponding enhancement values ranged from 47% to 580% after 30 days and from 28% to 366% after 60 days (eq. 7; Fig. 4). Considering the total wild population, the contribution of hatchery crabs ranged from 10% to 38% 30 days after release of hatchery crabs and from 8% to 36% 60 days after their release (Fig. 4). Averaged over the duration of sampling within each cove and over all four batches, the contribution of hatchery crabs to the total population was 17.4% (an enhancement level of 21.0%) and to the target population segment was 50.2% (an enhancement level of 100.7%). These values indicate that half of the crabs in this size range were of hatchery origin and that addition of hatchery crabs doubled this segment of the population.

Enhancement levels varied among the four batches of hatchery crabs (repeated-measures ANOVA, $F_{[3,18]} = 21.0$, $p < 0.001$ considering wild crabs in the hatchery size range, and $F_{[3,18]} = 15.9$, $p < 0.001$ considering all wild crabs). Enhancement of the two late-summer 2002 sites, Logan Pond and Sheepshead Cove, was higher than that of the sites of the two releases into Boathouse Creek in early summer 2002

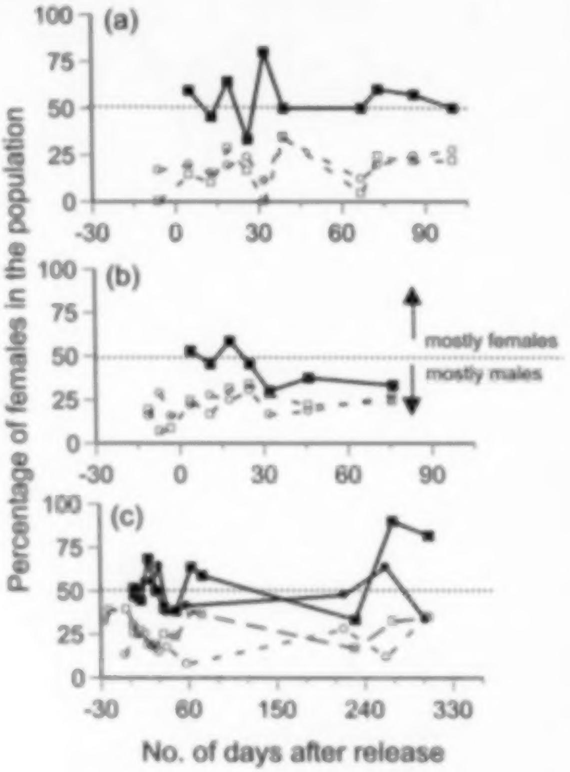


Fig. 7. Percentages of females in the hatchery group and the wild population over time for batch A (*a*), batch B (*b*), and batches C and D (*c*). The dotted line represents the scenario in which the number of males equals the number of females (—■—, hatchery batch A; —●—, hatchery batch B; - -□- -, wild (total) in batch C; - -○- -, wild (total) in batch D). In *a* and *b* the gender ratios for both the total wild population as well as just those wild crabs in the size range of the hatchery crabs are presented (—■—, hatchery; - -□- -, wild (hatchery size range); - -○- -, wild (total)). In *c*, for batches C and D, few wild crabs in the appropriate size range were present.

Fig. 8. Movement of hatchery crabs away from initial release areas in each enhancement cove. Each diagonal regression line represents the linear relationship between time and percentage of hatchery crabs in the release area. The horizontal dotted line represents the percentage of hatchery crabs that would be present in the release area if all were evenly distributed in the enhancement cove. The intersection of the regression line with this dotted line indicates that crabs have become evenly distributed. The batch letter is indicated on each regression line (—■—, batch A; —●—, batch B; - -□- -, batch C; - -○- -, batch D).

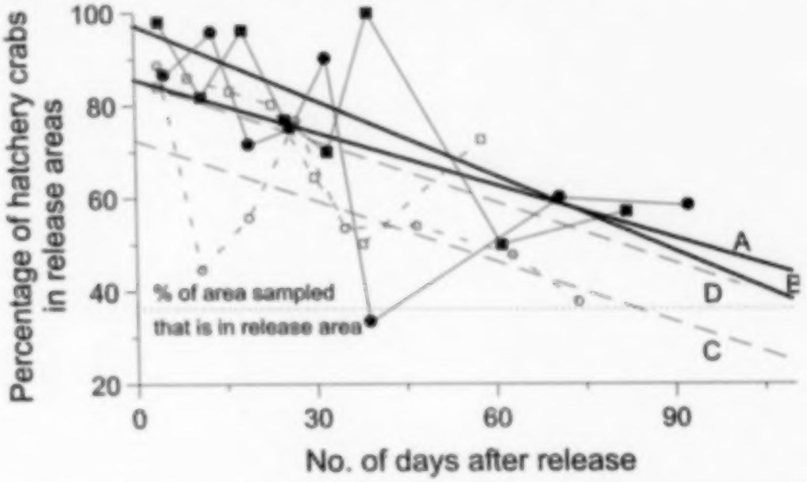


Table 2. Results of a two-way analysis of variance of a comparison of wild crab densities before and after (1 week and 2 weeks) the release of hatchery-raised juveniles.

	df group	df error	1 week after		2 weeks after	
			F	*p*	*F*	*p*
Total wild crabs						
Before vs. after release	1	155	12.5	**0.001**	4.5	**0.036**
Batch	3	155	5.2	0.002	7.4	<0.001
Interaction	3	155	1.6	0.193	3.8	0.011
Juvenile crabs only						
Before vs. after release	1	155	0.53	0.469	0.03	0.864
Batch	3	155	10.8	<0.001	10.4	<0.001
Interaction	3	155	0.06	0.980	0.1	0.940

Note: Boldface indicates statistical significance.

(Fig. 4). Considering just hatchery-sized crabs, contribution values were estimated to be 70% and 79% for the late-summer batches C and D, but only 33% and 22% for batches A and B, respectively. This difference was driven by lower wild crab abundances later in the summer when batches C and D were released, not by better survivorship of hatchery crabs in batches C and D than in batches A and B. Survivorship over time was not significantly different among the four batches (ANCOVA, effect of batch, $F_{[3,49]} = 0.8$, $p = 0.49$) (Fig. 5). After 60 days, mean survivorship of the four batches was 28 ± 7%. However, because late-released batches had to overwinter before reaching maturity, survivorship to size at maturity was higher for the first two batches (16%–20%, with maturity occurring 70–100 days after release) than for the last two batches (5%–15%, with maturity occurring 300–330 days after release) (Fig. 5).

Growth, gender ratio, and dispersal

Growth rates varied among batches (Fig. 6). The two batches released earlier in the summer had significantly higher growth rates than those released later in the summer (ANCOVA, effect of batch, $F_{[3,49]} = 6.6$, $p = 0.001$; Tukey's post-hoc tests, batches A,B ≠ C,D). Some crabs in the first two batches, A and B, grew to a size of 120 mm CW in 14 weeks (which is size at maturity; Hines et al. 1987) before recaptures ceased. Crabs in batch C reached a mean size of 46 mm CW in 11 weeks before the overwintering period. By April 2003, 9 months after release, these crabs were only 58 mm CW. Crabs in batch D only grew to a mean size of 33 mm CW in 8 weeks before winter, resuming growth in spring 2003 and requiring about a year to reach 120 mm CW (Fig. 6).

Hatchery crabs of each batch contributed a greater proportion of females to the blue crab subpopulations in their respective sites than did wild crabs (Fig. 7). Gender ratios of hatchery crabs were approximately 50:50 but differed from those expected from the wild crab population (χ^2 tests: χ^2 = 88, $p < 0.001$, for batch A; χ^2 = 136, $p < 0.001$ for batch B; χ^2 = 51, $p < 0.001$, for batch C; χ^2 = 116, $p < 0.001$, for batch D). For example, over the 15-week sampling period for batch A, 55.3% of all hatchery crabs caught were female, while only 23.0% of all wild crabs and 20.9% of just those wild crabs within the size range of hatchery crabs were female. In the case of batch B, the percentage of females was 53.7% for hatchery crabs, 22.9% for all wild crabs, and 23.0% for only those wild crabs within the size range of hatchery crabs. This gender discrepancy cannot be explained solely by habitat differences between maturing wild males and females (Hines et al. 1987), as it was apparent even when very small wild crabs <35 mm CW were considered.

Recaptures of hatchery crabs for several months after release into the four embayments revealed low dispersal away from release sites (Fig. 8). An even distribution of crabs would exist when the proportion of hatchery crabs caught in the release area was equal to the proportion of total sampling effort (area (m^2) sampled) concentrated in the release area. Only by 10–15 weeks after release did hatchery crabs approach an even distribution within the embayments (Fig. 8).

Displacement

Comparisons of densities in the four coves prior to hatchery releases with densities 1 and 2 weeks after release reveals that the density of wild crabs did not decrease as a result of the addition of hatchery juveniles. This suggests that displacement of wild crabs by hatchery crabs did not occur. Instead, in all four cases, the total number of wild crabs increased significantly (Table 2; Fig. 9). The number of wild juveniles, however, did not change significantly (Table 2).

Comparing wild crab densities in control sites before and after the release date enables determination of whether wild crab densities were increasing in other areas as well. If this were the case, an increase in wild crab density in enhancement sites would have been independent of the addition of hatchery crabs. However, in almost every control-site case, the change in density of wild crabs was either negative or not as positive as the change in wild crab density in the enhancement sites (Table 3; Fig. 10).

Discussion

The results from these relatively small-scale enhancement experiments suggest that it is possible to enhance local (sub)populations of blue crabs. We estimate that about 16%–20% of crabs released early in the summer survived to maturity, at the age of 5–6 months, and about 5%–15% of crabs released later in the summer survived to maturity, at the age of 12–13 months, both groups likely contributing to the overall spawning stock of Chesapeake Bay. Because an even ratio of hatchery females to males resulted, rather than the much lower ratio of female to male wild crabs (even very small juveniles) we observed in the field, the hatchery crabs may have made an even greater contribution to the next generation than an equal-number, but male-dominated, group of wild crabs maturing in these coves.

The growth rate of hatchery crabs released in early summer represented more than a 50% reduction in the time to maturity generally required by wild blue crabs in the Chesapeake Bay region. In Chesapeake Bay, wild crabs generally mature in 1.5 years (Fischler 1965; Hines et al. 1987). Larvae are released by wild females during the summer at the mouth of the Bay, followed by megalopal recruitment back into the Bay in the fall. Resulting juveniles must overwinter before resuming growth the next spring and reaching maturity by late summer. By bypassing the overwintering period and producing hatchery juveniles hatched in the spring, we were able to shorten the time to maturity, which in turn lessens the period during which crabs undergo mortality before producing offspring. However, hatchery juveniles produced and released later in the summer were forced, as wild juveniles are, to overwinter before reaching maturity; therefore, no advantage in time to maturity was achieved in those groups. We acknowledge that differences in growth between batches cannot be definitively attributed to season alone, as several other factors differed between the two early- and two late-summer releases. These factors included cove location and size, hatchery crab density, and fishing pressure (low in coves used for late-summer releases but appreciable in Boathouse Creek, as indicated by observations of trotline fishermen during summer).

Three processes could explain why we did not continue to recapture hatchery crabs after they reached maturity. That they had all died of natural causes is unlikely, as we estimated low mortality rates from week 4 to maturity. The second potential explanation is that mature crabs emigrated from our experimental sites. At this size, blue crabs in the study area exhibit a significant amount of emigration from sampling sites as females move down-bay to spawn (Hines et al. 1987, 1995). Third, mature crabs might have been lost to the fishery. For example, as many as 50–100 crabs, some potentially of hatchery origin, were removed from Boathouse Creek per day, which constitutes a significant proportion of the 1200–4000 crabs estimated to occupy the cove at any time.

Difficulty in judging success

One of the goals of these enhancement experiments was to begin to assess whether larger scale enhancement programs might prove fruitful. However, because quantitative studies of enhancement efforts are relatively new, though very much needed (e.g., Bannister et al. 1994; Heppell and Crowder 1998; Leber 1999; Crowe et al. 2002), objective

Fig. 9. Wild crab densities before hatchery crab release (open bars), 1 week after hatchery crab release (solid bars), and 2 weeks after hatchery crab release (hatched bars). Densities presented are not corrected for gear efficiency.

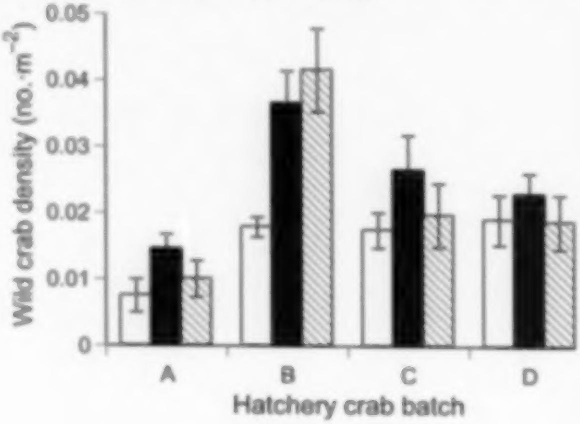


Table 3. Comparison of the change in wild crab densities after the release of hatchery-raised juveniles in control sites versus experimental sites.

Batch	No. of days between "before" and "after" sampling	No. of days after release[a]	Change in density (no.·m^{-2})[b] Enhancement sites	Control sites	t	p
B	14	11	+0.0246	+0.0002	3.3	0.002
C	25	6	+0.0119	+0.0039	1.3	0.192
D	34	16	+0.0062	–0.0241	5.0	<0.001

[a] Time elapsed between release of hatchery-raised crabs and "after" sampling.

[b] The change in wild crab density ("after" sampling minus "before" sampling) in both enhancement and control sites.

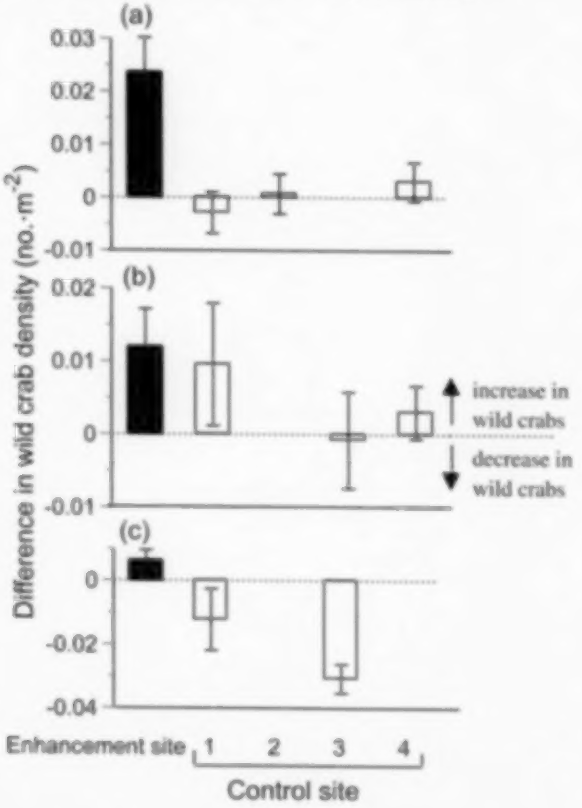


Fig. 10. Differences in wild crab density before and after release of hatchery crabs at enhancement and control sites for (*a*) batch B, (*b*) batch C, and (*c*) batch D. Control site 1 is the Rhode River from trawl samples; control site 2 is Logan Pond (before it was used as a release site); control site 3 is Boathouse Creek (1–2 months after it was used as a release site); and control site 4 is Cheston Creek. Densities used to calculate differences ("after" minus "before") were not corrected for gear efficiency.

standards have not yet been established against which to measure our results.

It has been suggested that the ultimate test of enhancement success lies in determining whether a hatchery is economically sustainable (Hilborn 1998), which, in turn, is measured by calculating the number and value of hatchery individuals appearing in the fishery. However, many benefits of enhancement, other than increased fishery catch, are difficult to judge in an economic context. In particular, enhancement programs may result in improvement of the reproductive output of a population by way of hatchery individuals surviving to maturity (Crowe et al. 2002). Therefore, while the economic value of a landed hatchery-produced mature female can be easily calculated, the unlanded hatchery-produced mature female, which is the goal of this program, may have a much amplified value through the spawning stock – recruitment relationship (Bannister et al. 1994; Bannister and Addison 1998; Crowe et al. 2002). These noncaught hatchery-produced individuals, which have economic value that is not easily estimated (because of the uncertainty of their reproductive contribution and the time lags associated with generation times) may be the most biologically valuable products of enhancement programs. Assessing the success of enhancing the reproductive output of a depressed or endangered stock is likely to be much more difficult, time-consuming, and complicated than estimating the direct transfer of hatchery-raised individuals into the fishery.

Relatively few other studies are available for comparison of enhancement levels. Many that do exist report recovery or recapture rates (Stoner and Glazer 1998), which aid in economic analyses but are inherently linked to the amount of sampling effort. This effort is usually variable even across studies based on fishery-independent data. As a result, recapture rates only provide a minimum survivorship rate over a minimum period of time. In addition, if recovery rates are calculated from fishery landings rather than research surveys, those values become mortality rates, providing little information about the number of individuals that survive to contribute to the brood stock.

Some reports do provide difficult-to-calculate survivorship rates for hatchery-produced individuals. However, because survivorship values for wild individuals in many cases are not known (Davenport et al. 1999), assessing the relative quality of these survivorship rates is not possible. In addition, comparisons across taxa are complicated by differences in life history or fishery patterns. For example, one might compare survivorship rates for two species over similar periods of time, but it might make more sense to compare survivorship rates over equal proportions of time to maturity or time to harvest. Most European lobsters (*Homarus gammarus*) take 4–5 years to reach maturity and legal size (Bannister et al. 1994; Bannister and Addison 1998), whereas blue crabs take 0.5–1.5 years. Comparisons of enhancement efforts must therefore account for this life-history difference.

Table 4. Contribution values (proportion of all individuals composed of hatchery-raised individuals) reported in other studies.

	Contribution (%)	Time (years)[a]	Reference
American lobster	0	1	Castro et al. 2001
Topshell	0	1.5	Crowe et al. 2002
Masu salmon	10.6	Recruitment to fishery	Masuda and Tsuakamoto 1998
Barramundi	15–20	3	Rimmer and Russell 1998
European lobster	10–35	4–8	Bannister et al. 1994
European lobster	26	4–8	Bannister and Addison 1998; van der Meeren et al. 1998
Scallops	40–50		Bull 1993
Red sea bream	0–50		Tsukamoto 1989; Imai et al. 1994; Masuda and Tsukamoto 1998
Threadfin	0–64	0.67	Leber et al. 1998
Chum salmon	90		Kaeriyama 1996

[a] Time after release at which contribution was measured, where available.

Attempting to judge success

Despite the challenges posed to assessment, comparisons with other efforts suggest that our experiments provided relatively high levels of enhancement. We estimated 5%–20% survivorship to maturity for blue crabs, higher than that reported for several other species. Survivorship was estimated at 0.0007% for released cod (*Gadus morhua*) larvae resampled after 1 year, a period shorter than the time to maturity (Svåsand 1998). Only 1%–3% of hatchery-produced abalone, *Haliotis rufescens* (which mature after 2–3 years), survived after 1 year (Tegner and Butler 1985, 1989). Survivorship of queen conch (*Strombus gigas*), which mature after 3 years, was 0.0005%–1.4% per year (Stoner and Glazer 1998). Of stocked panaeid shrimp, 2.8%–3.5% survived to fishable size at the age of 6 months (Davenport et al. 1999). After 1 year, survival of topshell, *Trochus niloticus*, which reach maturity at 2–3 years, was 4.4% (Crowe et al. 2002).

Some studies provide survivorship values equal to or higher than those we estimated for blue crabs. In Texas, 21% of red drum, which recruit to the fishery at the age of 1–2 years, survived to age 2 (McEachron et al. 1998). At least 10–30% of Japanese flounder survived to fishery size (Masuda and Tsukamoto 1998). Though recapture rates in European lobster fisheries, when hatchery individuals were 4–8 years old, were only <2% (Cook 1995; Bannister and Addison 1998), survivorship of these hatchery individuals was estimated at 37%–80% (Bannister et al. 1994; Bannister 2000). The discrepancy between recapture rate and survival rate indicates that a large proportion of hatchery individuals escaped fishing mortality and potentially contributed reproductively to the population.

Quantifying survivorship is only a piece of determining enhancement success, as in many cases, baseline survivorship rates of wild juveniles are not known. Determining the degree to which hatchery individuals contribute to the total population can help, despite the fact that because a second density estimate is required (that of wild individuals), these values can be very variable (Table 4). Both terms, survivorship and contribution, have merits and negatives and should be considered together when assessing success. There are cases in which survivorship of hatchery animals is low, but because wild densities are also low, contribution is high. There may also be cases in which survivorship of hatchery animals is high, but because few hatchery individuals are released, contribution may be low. In our experimental releases, densities of hatchery and wild crabs were generally relatively balanced as a result of substantial hatchery crab survivorship, resulting in a high contribution.

Large-scale stock enhancement of the swimming crab in Japan may provide the best available comparison with the blue crab (summarized in Secor et al. 2002). During the 1990s, Japanese hatchery releases ranged from 700 000 to 1 400 000 juveniles per year in three bay systems ranging from 10 000 to 100 000 ha. Harvest recapture rates ranged from 1.2% to 31.3%, and hatchery contribution to the fishery catch ranged from 9% to 59% (Ariyama 2000; Secor et al. 2002). However, despite the large scale of hatchery releases, experimental analysis to quantify survival and contribution of hatchery swimming crabs to the wild population has been limited by lack of replication (Secor et al. 2002).

Carrying capacity

When assessing enhancement success, ultimate effect on population size must also be considered. If populations are limited not by recruitment but by resource availability, hatchery individuals may survive and appear in significant proportions in populations, but this may not lead to larger population sizes. Instead, they may be displacing wild individuals, either through direct competition or by indirectly using limited resources. Displacement can be assessed, as in our study, with paired measurements of densities in both enhanced and control areas coupled with tagging of hatchery individuals (Heppell and Crowder 1998; Hilborn 1998; Crowe et al. 2002).

Our results indicate that replacement of wild crabs by hatchery crabs did not occur. Lack of displacement of wild crabs and low dispersal of hatchery crabs away from release sites suggest that total crab densities in enhancement coves were below carrying capacity. Rather than serving the role of out-competitor, hatchery crabs may instead have served the role of potential prey for wild crabs, as blue crabs are highly cannibalistic. Wild crabs appeared to be drawn into our enhancement sites immediately after release of hatchery crabs, perhaps attracted to our released hatchery crabs as prey.

Others have suggested that current blue crab population levels are below carrying capacity in many areas of the Chesapeake Bay (Lipcius and Stockhausen 2002). Wild densities measured in our enhancement coves ranged from 0.004

to 0.07 crabs·m^{-2} (40–720·ha^{-1}), much lower than the densities reported for similar types of systems during historical highs of blue crab abundance in the Chesapeake Bay (Hines et al. 1987). Our enhancement efforts resulted in an initial increase in density by 0.07–0.33 crabs·m^{-2} (700–3300 ha^{-1}), which, even when added to the wild populations already present, falls below probable carrying capacity densities.

In conclusion, our experimental enhancements of local blue crab populations met several of the criteria for success articulated in recent years (Hilborn 1998; Leber 1999). Tagging enabled us to determine that hatchery crabs contributed to the populations of our small embayments, doubling, on average, the number of crabs in the hatchery crab size range and increasing the total wild population by a third. Tagging also enabled us to determine that hatchery crabs released earlier in the summer matured in a much shorter period than expected for wild crabs. The use of control sites enabled us to determine that hatchery crabs did not displace wild crabs, which suggests that these areas are below carrying capacity.

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Stable carbon and nitrogen isotope analysis of *Nephrops norvegicus / Merluccius merluccius* fishing grounds in the Bay of Biscay (Northeast Atlantic)

François Le Loc'h and Christian Hily

Abstract: The trophic structure of the benthic ecosystem of the Grande Vasière (Great Mud Bank), a heavily trawled area in the Bay of Biscay, is largely unknown. To better understand the biotic interactions between exploited species (mainly Norway lobster, *Nephrops norvegicus*, and European hake, *Merluccius merluccius*) and their competitors and prey, we applied a dual stable carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope analysis to determine trophic levels and differences in the potential food sources of species in the benthic and demersal communities. Five main groups of species constitute the basis of the trophic structure. They are distributed in three main trophic levels: primary consumers; secondary consumers including *N. norvegicus* and juvenile *M. merluccius*; and top predators including adult *M. merluccius* ($\delta^{15}N$ = 14.5‰). The large differences in $\delta^{13}C$ values within the primary consumers attest to two different food components: a pelagic component composed of fresh sedimenting particulate organic matter, which mainly supplies supra- and epi-benthic suspension feeders, and zooplankton, and a benthic component, which mainly supplies deposit feeders. For the predators, the target species, and their competitors, diet changes during the life-span are reflected by differences in isotope values for the size classes.

Résumé : La structure trophique de l'écosystème benthique de la Grande Vasière, une importante zone de pêche au chalut de fond du Golfe de Gascogne, demeure encore méconnue. Afin de mieux comprendre les interactions biotiques entre les espèces exploitées (principalement la langoustine, *Nephrops norvegicus*, et le merlu, *Merluccius merluccius*) et leurs concurrents et proies, une analyse des isotopes sables du carbone ($^{13}C/^{12}C$) et de l'azote ($^{15}N/^{14}N$) a été réalisée pour étudier les différents niveaux trophiques et mettre en évidence l'existence de différentes sources de nourriture potentielles des communautés benthiques et démersales. Cinq principaux groupes trophiques constituent la base de la structure de la chaine alimentaire. Trois niveaux trophiques ont été identifiés : consommateurs primaires, consommateurs secondaires (*N. norvegicus* et les juvéniles de *M. merluccius*) et les supers prédateurs comme les adultes de *M. merluccius* ($\delta^{15}N$ = 14,5 ‰) Les grandes différences de $\delta^{13}C$ au sein des consommateurs primaires attestent de deux différentes sources de nourriture : l'une pélagique composée de matière organique particulaire fraiche sédimentant, qui alimente principalement les suspensivores supra- et épi-benthiques, et le zooplancton et l'autre benthique qui alimente les déposivores. Les changements de régimes alimentaires des prédateurs, l'espèce cible et leurs compétiteurs, au cours de leur vie, se traduisent par des différences isotopiques entre les différentes classes de taille.

Introduction

The impact of benthic trawling on macrofaunal communities has been studied in terms of changes in abundance and biomass (Jennings and Kaiser 1998; Lindeboom and de Groot 1998; Kaiser et al. 2000) and in the structure of the marine food web (Pauly et al. 1998; Jennings et al. 2001). Benthic trawling leads to modifications of trophic interactions (predation and competition) through species removal (target and bycatch species), direct and indirect mortality (stimulation of scavenging and opportunistic predation), and mechanical disturbance of benthic habitats (reviewed by Jennings and Kaiser 1998).

The continental shelf of the Bay of Biscay is an important demersal and benthic fishery for France. In its northern part, from southern Brittany to the Loire estuary, the Grande Vasière (Great Mud Bank) constitutes a main fishery for European hake, *Merluccius merluccius* (hereinafter referred to as hake) and Norway lobster, *Nephrops norvegicus*. Benthic and demersal fish communities of the Bay of Biscay have recently been described (Poulard and Boucher 1997; Blanchard 2000), but no work since that of Glémarec (1969) has investigated macroinvertebrate benthic communities on a

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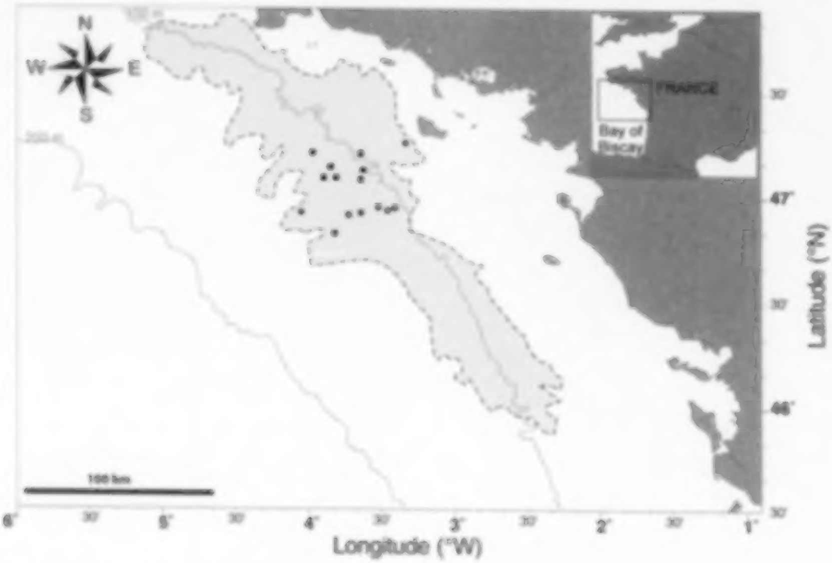
Fig. 1. Western part of the continental shelf of the Bay of Biscay, northeast Atlantic Ocean, showing the Great Mud Bank (within the broken line) and sampling stations (■).



large spatial scale. The aims of this study were to better understand the trophic position of two species of economic importance (*M. merluccius* and *N. norvegicus*) in their respective communities and, in particular, to identify their main trophic competitors and prey. To investigate this question, we used stable nitrogen isotope ratios as an indicator of trophic level and $\delta^{13}C$ as a tracer of the origin of the food consumed.

Indeed, the heterotrophic stable-isotope signature depends on the stable-isotopic signatures of the prey and on isotopic fractioning during food processing. Stable nitrogen and carbon isotope ratios (expressed as $\delta^{15}N$ and $\delta^{13}C$, respectively) are typically increased from prey to consumers by 3‰–4‰ for $\delta^{15}N$ and 1‰ for $\delta^{13}C$ (DeNiro and Epstein 1981; Minagawa and Wada 1984; Cabana and Rasmussen 1994). Thus, $\delta^{15}N$ can indicate the trophic position of an organism within the food web on the basis of the organic matter and the primary producers it consumes. The particular $\delta^{13}C$ values found in primary producers (phytoplankton and microphytobenthos) are also responsible for differences in the isotopic signatures of their consumers (Riera et al. 1999), allowing distinctions to be made between pelagic and benthic food webs (Hobson et al. 2002). Consequently, $\delta^{13}C$ can be used as a tracer of organic trophic sources for benthic consumers (Peterson 1999).

Materials and methods

Study area

A large sedimentary bank known as the Grande Vasière or Great Mud Bank characterizes the northern continental shelf of the Bay of Biscay (French coast, northeast Atlantic Ocean) (Fig. 1). It is situated at a depth range of 80–130 m and covers a distance of 275 km from south to north and 55–75 km from east to west. It consists of a large expanse of muddy sediments lying parallel to a coastal area composed of prelittoral depressions, gravelly plains, and hydraulic dunes. To the west, at a depth of around 130 m, a hard bottom separates the Great Mud Bank from the outer edge of the continental shelf, which is composed of sands containing *Ditrupa arietina* down to 160 m depth (Glémarec 1971). The Great Mud Bank is the location of an important French demersal and benthic fishery, mainly for hake and Norway lobster, but also for sole (*Solea solea*) and anglerfish (*Lophius* sp.) (Dardignac 1988).

The study area (sandy mud 95–120 m in depth) selected was in the central part of the hake fishery. The soft-bottom macrofauna was defined by Glémarec (1969) as a *Nucula sulcata* – *Brissopsis lyrifera* community.

Sampling

All data used for isotopic analysis were collected from 10 sites within the central part of the Great Mud Bank selected to represent the variability of the muddy sediment on the basis of previous data (Chassé and Glémarec 1976). Analysis of the sediment at our sampling stations showed that the pelitic fraction (i.e., the fraction of the sediment with particle sizes <63 µm) ranged from 9.65% (median particle size 170–200 µm at 127 m depth) to 34.86% (median particle size 170–200 µm at 110 m depth). A 2-m long beam trawl was used in October 2000 to sample the epibenthos, i.e., invertebrate megafauna (individual mean length >10 mm) and demersal fish (Kaiser et al. 1994; Jennings et al. 1999; Ellis et al. 2000). This proved to be an efficient method for catching large and rare species and for integrating small-scale seabed patchiness (Frauenheim et al. 1989). The beam trawl was fitted with a chain mat and a 20 mm mesh liner. During sampling (20 min), warp length was three times water depth, and the distance trawled was measured by an onboard differential global positioning system. The benthic macrofauna (individual mean length 1–10 mm) was sampled using a Hamon grab (0.25 m^2) in October 2000. A suprabenthic sledge (Macer-GIROQ) was used for suprabenthos collection (0.5 mm mesh net) and zooplankton was sampled for the entire water column with a WP2 zooplankton net (0.2 mm mesh) in May 2001. Fish samples were obtained with an otter trawl in December 1999.

At four stations, particulate organic matter (POM) was collected with a Niskin bottle twice a day during 4 days in September 2002 at two depths. The first depth was near the fluorescence maximum at approximately 30 m (above the thermocline, which was close to 40 m) depending on the station and the time of day, and the second was close to the bottom at a distance of 1–2 m from the sediment. They were termed the surface-layer POM and bottom POM, respectively. As a result of technical constraints on field cruises in these offshore waters, not all the samples were taken during the same period. The bias induced by this problem was taken into account in the analysis of results.

Stable isotopes

POM was collected by filtering seawater (4 L was filtered for each sample) on precombusted Whatman GF/F filters and then stored at −20 °C. Subsequently, the filters were exposed to HCl vapor for 4 h to remove carbonates before being placed in tin cups. The samples were analyzed for isotope ratios using a Finnigan Delta S isotope-ratio mass spectrometer coupled to a Carlo Erba NA 2100 element analyzer. Because of the low organic-matter content (<2%) and the high levels of carbonates measured in the sediment, total extraction of the sediment organic matter without degradation was not possible; therefore isotopic analysis of the sediment organic matter was not carried out.

Species selected for isotopic analysis were those that were dominant in both abundance and biomass and also accessible for predation by the two target species. Zooplankton and suprabenthos samples were acidified (10% HCl) to remove any residual carbonates from cuticles and then rinsed with distilled water (Riera et al. 2000). Mega- and macro-faunal (except polychaete) muscle tissue was used for stable-isotope analysis. Muscle samples were taken from the dorsal musculature of the fishes, from pereiopod 1 of the Brachyura, from the abdomen of the Natantia, Reptantia, Macrura, Stomatopoda, and large Mysidacea, and from the siphon of the bivalves. The polychaetes' viscera were extracted and the analyses carried out on the remaining whole body. After dissection, the tissue samples from all the taxa were washed very carefully with distilled water to prevent any contamination by the sediment carbonates (Kharlamenko et al. 2001; Jennings et al. 2002; O'Reilly et al. 2002).

All samples were stored, individually frozen, at −20 °C before freeze-drying. Each dried sample was then ground to a homogeneous powder, and 1 mg of this powder was weighed in tin capsules for stable carbon and nitrogen isotope analyses. The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of faunal tissue were analysed by continuous-flow isotope-ratio mass spectrometry using a Europa Scientific ANCA-NT 20–20 stable-isotope analyzer together with a ANCA-NT solid–liquid preparation module. As the samples contained more than 10% nitrogen, the continuous-flow isotope-ratio mass spectrometer was operated in dual-isotope mode, allowing $\delta^{15}N$ and $\delta^{13}C$ to be measured in the same sample. Analytical precision (standard deviation, $n = 5$) was 0.2‰ for both nitrogen and carbon, as estimated from standards analyzed together with the samples. Stable-isotope ratios were expressed in conventional δ notation as parts per mil (‰) according to the following equation:

$$(1)\quad \delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where X is ^{13}C or ^{15}N and R is the corresponding $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio, respectively.

As $\delta^{15}N$ values provide an indication of the trophic position of a consumer, the following formula was used to estimate trophic level:

$$(2)\quad \text{trophic level} = (\delta^{15}N_{\text{consumer}} - \delta^{15}N_{\text{mean POM}})/3.4 + 1$$

where 3.4‰ is the assumed ^{15}N trophic-enrichment factor according to Minagawa and Wada (1984). In a benthic ecosystem such as the Great Mud Bank (depth >100 m), no primary production can occur on the bottom; consequently, the only organic material available for benthic primary consumers is assumed to be the POM sedimenting from upper water layers and therefore this is designated as the first trophic level.

Statistical methods

Nonparametric comparative tests (Mann–Whitney U test and Kruskal–Wallis H test) were carried out to determine differences between isotope values. Nonparametric tests were chosen for all species because of small sample sizes and the non-normal distribution of most of the data. Spearman's rank-correlation coefficients were used to measure relationships between stable isotope values and increasing length. A hierarchical cluster analysis using Ward's minimum-variance method was performed on stable nitrogen and carbon isotope values to identify groups of species (Davenport and Bax 2002).

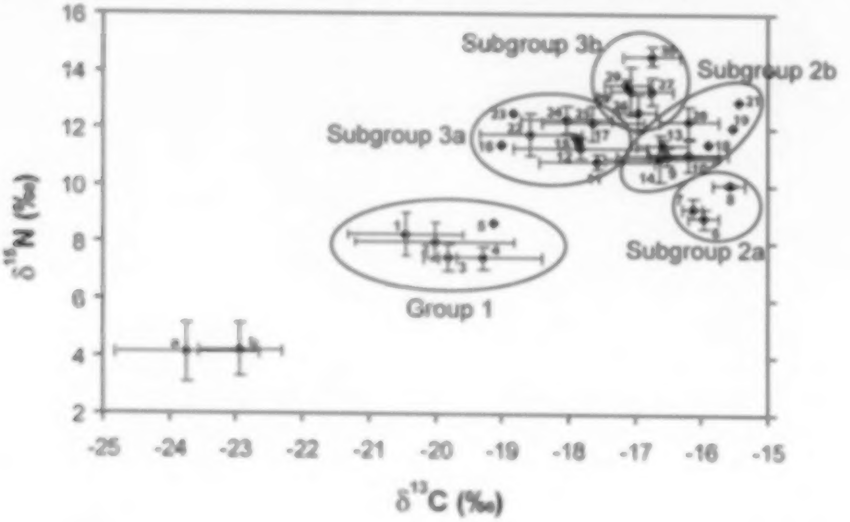
Results

Identification of trophic groups

Stable nitrogen isotope ratios ranged from 4.1‰ (surface-layer POM) to 14.5‰ (*M. merluccius*), corresponding to four main trophic levels. Analysis showed a wide range of $\delta^{13}C$ signatures, from −23.7‰ (surface-layer POM) to −15.4‰ (the crab *Goneplax rhomboides*). Like $\delta^{15}N$, $\delta^{13}C$ showed no significant difference between surface-layer POM and bottom POM (Mann–Whitney U test, $n = 4$ for both depths, $p = 0.39$ and $p = 0.06$ for $\delta^{15}N$ and $\delta^{13}C$, respectively), but a number of benthic organisms (bivalves, polychaetes, crustaceans) were significantly enriched in ^{13}C compared with some pelagic organisms (*Crangon echinulatus* and *Lophogaster typicus*) and benthic suspension feeders (*Ascidia* sp. or *Callianassa subterranea*) (Mann–Whitney U test, $n = 10$ and $n = 34$ for the pelagic and suspension-feeding organisms and the other benthic invertebrates, respectively; $p < 0.001$). There was also a continuum of $\delta^{13}C$ values in each taxonomic group.

The hierarchical cluster analysis identified three main groups of species, two of which were divided into two subgroups, represented in a dual-plot graph in Fig. 2. The first assemblage of five taxa (group 1) was clearly separated. This group is composed of benthic suspension feeders, zooplank-

Fig. 2. Distribution of carbon and nitrogen stable isotope ratios (mean ± standard deviation) among groups composing the Great Mud Bank food web. The groups of taxa (encircled) were chosen by are the result of hierarchical cluster analysis (the number of samples is given in parentheses). *a*: particulate organic matter at –30 m depth (8); *b*: particulate organic matter at the bottom (8); group 1: 1 zooplankton (4), 2 suprabenthos (4), 3 *Ascidia* sp. (4), 4 *Callianassa subterranea* (3), and 5 *Hyalinoecia fauveli* (1); subgroup 2*a*: 6 *Aequipecten opercularis* (5), 7 *Nucula sulcata* (6), and 8 *Alpheus glaber* (4); subgroup 2*b*: 9 *Nephrops norvegicus* (32), 10 *Munida sarsi* (22), 13 *Crangon allmani* (5), 14 *Notomastus latericeus* (8), 18 *Nephtys caeca* (1), 19 *Goneplax rhomboides* (1), 20 *Liocarcinus depurator* (6), and 21 *Glycera rouxii* (1); subgroup 3*a*: 11 *Terebellides stroemi* (4), 12 *Lumbrineris impatiens* (3), 15 *Processa nouveli* (1), 16 *Crangon echinulatus* (1), 17 *Sepia* sp. (1), 22 *Merluccius merluccius* (8–15 cm long, 13), 23 *Lophogaster typicus* (1), 24 *Trachurus trachurus* (13), and 25 *Micromesistius poutassou* (10); subgroup 3*b*: 26 *Callyonimus lyra* (4), 27 *Squilla desmaresti* (4), 28 *Trisopterus minutus* (12), 29 *M. merluccius* (19–36 cm, 20), and 30 *M. merluccius* (39–47 cm, 10).



ton, and suprabenthic crustaceans (trophic level close to 2 and $\delta^{13}C$ close to –20‰).

A second assemblage (subgroup 2*a*) is composed of deposit feeders (two bivalves and a burrowing crustacean) with a trophic level close to 2.5 ($\delta^{15}N$ = 8‰–10‰) and strong enrichment in ^{13}C ($\delta^{13}C$ close to –16‰) compared with the suspension feeders. Subgroup 2*b* (trophic level 3.0–3.6 and $\delta^{13}C$ around –16‰) is composed of benthic organisms: a subsurface deposit-feeding polychaete (*Notomastus latericeus*), carnivorous decapod crustaceans (*N. norvegicus, Munida sarsi, Liocarcinus depurator, G. rhomboides*), carnivorous peracarid crustaceans (*Crangon allmani*), and polychaetes (*Nephtys caeca, Glycera rouxii*). Their ^{13}C composition allowed their distinction from the suspension feeders, hence these two subgroups are identified as the benthic food-web component.

The third group is composed of secondary consumers consisting of species from the pelagic and demersal food-web component, primarily because of their food affinities (all species had $\delta^{13}C$ below –16.7‰), and secondarily because of their trophic level. The trophic level of the analyzed species ranged from 3 to 3.7, suggesting a mixed diet based on several different trophic levels. In subgroup 3*a*, the pelagic food-web component is composed of species with $\delta^{13}C$ averaging –19‰ to –18‰. These are the "true" pelagic feeders, i.e., the fish *Trachurus trachurus*, the cephalopods *Sepia* sp., and some Crustacea, Mysidacea, and peracarid shrimps (*C. echinulatus, Processa nouveli*). The top predators, subgroup 3*b*, which reached the fourth trophic level (3.6–4.0), had $\delta^{15}N$ levels up to 14‰. These predators are not only demersal fishes such as the adult hake, but also benthic crustaceans such as *Squilla demaresti*.

The target species, *M. merluccius* and *N. norvegicus*, and their competitors

Each individual $\delta^{13}C$ and $\delta^{15}N$ value was plotted against size for the two main exploited species, *M. merluccius* and *N. norvegicus* (Figs. 3 and 4). Correlation coefficients were used to explore relationships between size (total length for fishes and cephalothorax length for crustaceans) and stable-isotope composition (Table 1). There is ^{15}N and ^{13}C enrichment in hake muscle with increasing size (r_s = 0.906, $p <$ 0.001 for $\delta^{15}N$; r_s = 0.803, $p < 0.001$ for $\delta^{13}C$). Three size classes corresponding to age (Pinero and Sainza 2003) can be statistically differentiated, 9–15 cm (first year), 19–36 cm (1–3 years old), and 39–47 cm (4–5 years old), for both isotopes (Mann–Whitney *U* test, $p < 0.01$ for $\delta^{13}C$ and $\delta^{15}N$; Table 2), with an increase of 1.87 for $\delta^{13}C$ and 3.2–4.0 for trophic level. *Nephrops norvegicus* exhibits a slight but significant relation between size and $\delta^{15}N$ (r_s = 0.564, $p < 0.001$), with the trophic level remaining close to 3.0 (enrichment = 0.53‰) from shorter to longer size classes. Conversely, a strong increase of $\delta^{13}C$ with size was found (enrichment = 1.65‰, r_s = 0.889, $p < 0.001$). As for hake, the $\delta^{13}C$ values for the three size classes defined, 14–17 mm (first year), 22–34 mm (2–5 years old), and 39–42 mm (6–7 years old)

Fig. 3. Relation between muscle and size (total length) for Atlantic hake, *Merluccius merluccius* (n = 43), for $\delta^{15}N$ (*a*) and $\delta^{13}C$ (*b*).

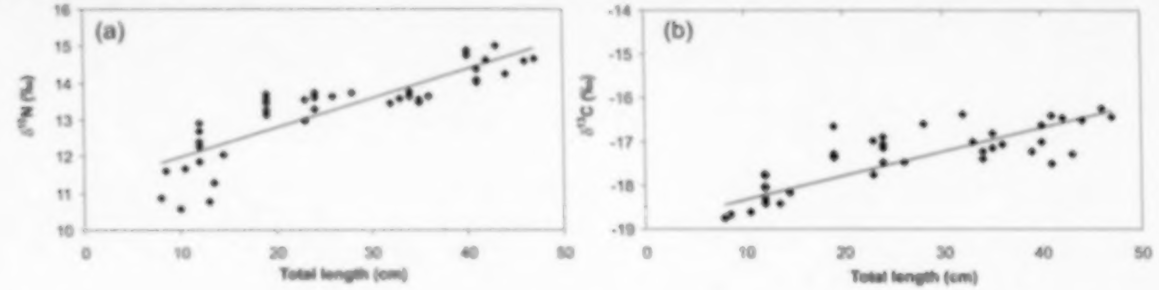


Fig. 4. Relation between muscle and size (cephalothorax length) for Norway lobster, *Nephrops norvegicus* (n = 32), for $\delta^{15}N$ (*a*) and $\delta^{13}C$ (*b*).

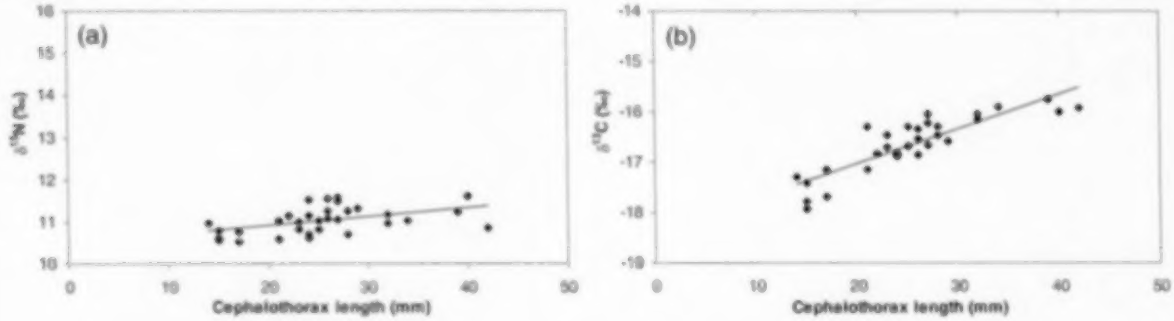


Table 1. Correlation between $\delta^{15}N$ or $\delta^{13}C$ values and size (total length for fishes and cephalothorax length for crustaceans).

		$\delta^{15}N$		$\delta^{13}C$	
	n	r_s	p	r_s	p
Merluccius merluccius	43	**0.906**	<0.001	**0.803**	<0.001
Nephrops norvegicus	32	**0.564**	<0.001	**0.889**	<0.001
Munida sarsi	22	**0.635**	<0.001	**0.661**	<0.001
Trisopterus minutus	12	**0.796**	<0.001	0.246	0.209
Micromesistius poutassou	10	0.022	0.473	0.468	0.063
Trachurus trachurus	13	0.521	0.034	-0.028	0.464

Note: Values in boldface type are significant.

(Verdoit et al. 1999), are statistically different (Mann–Whitney test, $p < 0.01$; Table 2).

Analysis was also conducted for the by-catch species *M. sarsi* (Fig. 5), *Micromesistius poutassou*, *Trisopterus minutus*, and *T. trachurus* (Fig. 6). *Munida sarsi* displayed a similar trend to *N. norvegicus* for both carbon and nitrogen. The $\delta^{15}N$ and $\delta^{13}C$ values showed a significant correlation and low variability with size ($r_s = 0.635$, $p < 0.001$, standard error = 0.84‰, and $r_s = 0.661$, $p < 0.001$, standard error = 0.74‰, for $\delta^{15}N$ and $\delta^{13}C$, respectively). Comparison of the isotope ratios for each of the three size classes of *N. norvegicus* with those of *M. sarsi* reveals no significant differences for either isotope, the only exception being the comparison of $\delta^{13}C$ values for the shorter size classes (Mann–Whitney U test, $p = 0.002$). *Micromesistius poutassou* and *T. trachurus* displayed no correlation with increasing size for either $\delta^{15}N$ or $\delta^{13}C$ (Table 1). In the results for *T. minutus* there was no correlation between $\delta^{13}C$ values and individual size, whereas a strong correlation existed between $\delta^{15}N$ values and size ($r_s = 0.796$, $p < 0.0001$).

Discussion

No significant differences appeared in the stable-isotope composition of surface-layer and bottom POM. This similarity indicates that during the September period POM reached the benthic ecosystem without isotopic changes and this concurs with the observations of Hobson et al. (1995). Our $\delta^{15}N$ values for POM are in agreement with those reported by Fry (1988) for Georges Bank, by Hobson et al. (1995) for living phytoplankton in the North East Water Polynya Project, by Kang et al. (1999) and Riera et al. (1999) for the coastal

Table 2. Nitrogen and carbon stable isotope ratio statistics for *Merluccius merluccius*, *Nephrops norvegicus*, and *Munida sarsi*, showing numbers of measurements (*n*), median isotope values, the differences between successive size-class categories (enrichment), and the significance of the differences (Mann–Whitney *U* test; ns, not significant), and trophic levels.

		$\delta^{15}N$				$\delta^{13}C$		
Taxon and size class	*n*	Median (‰)*	Enrichment (‰)	*p*	Trophic level	Median (‰)*	Enrichment (‰)	*p*
Merluccius merluccius								
9–15 cm	13	11.84 (1.27)	—		3.2	−18.42 (0.60)	—	
19–36 cm	20	13.59 (0.19)	1.74	<0.001	3.7	−17.14 (0.44)	1.44	<0.001
39–47 cm	10	14.62 (0.54)	1.01	<0.001	4.0	−16.52 (0.82)	0.43	0.009
Nephrops norvegicus								
14–17 mm	6	10.68 (0.25)	—		2.9	−17.55 (0.50)	—	
22–34 mm	23	11.04 (0.44)	0.38	0.002	3.0	−16.51 (0.53)	1.04	<0.001
39–42 mm	3	11.22 (0.76)	0.15	ns	3.1	−15.93 (0.25)	0.61	0.005
Munida sarsi								
14–19 mm	6	10.59 (0.32)	—		2.9	−16.71 (0.50)		
20–24 mm	12	11.12 (0.56)	0.29	ns	3.0	−16.38 (0.61)	0.20	ns
26–29 mm	4	11.57 (0.26)	0.55	0.005	3.2	−15.86 (0.44)	0.54	0.017

*Values in parentheses are interquartile ranges.

Fig. 5. Relation between muscle and size (cephalothorax length) for *Munida sarsi* (*n* = 22) for $\delta^{15}N$ (*a*) and $\delta^{13}C$ (*b*).

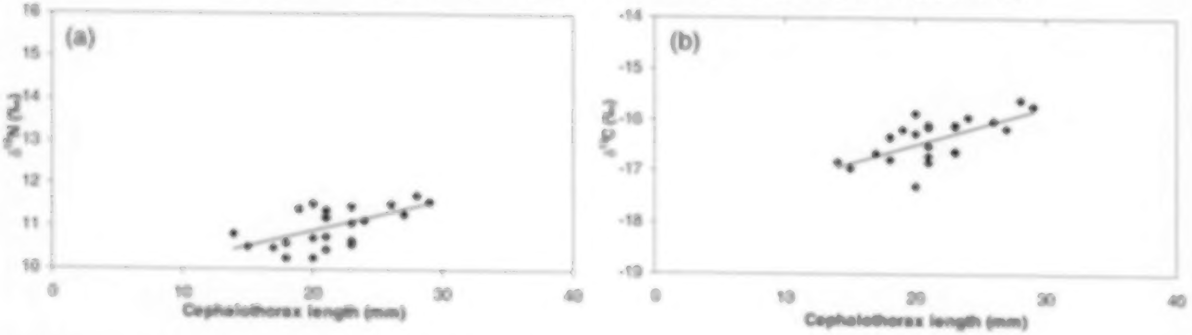
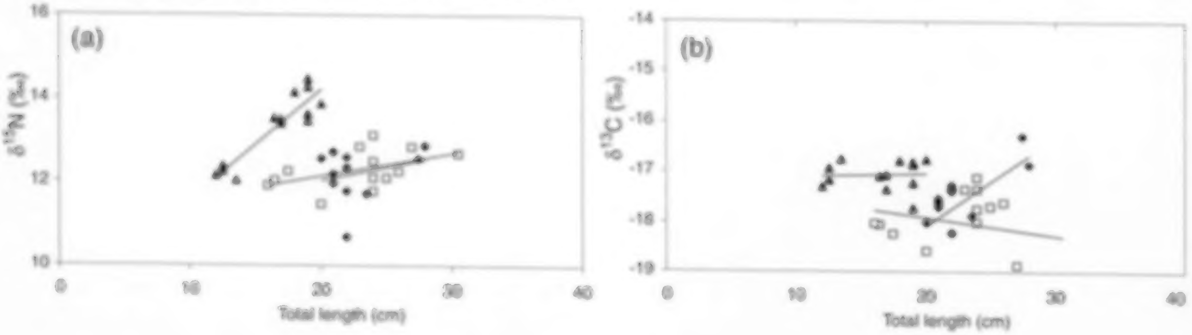


Fig. 6. Relation between muscle and size (total length) for *Micromesistius poutassou* (◆) (*n* = 10), *Trisopterus minutus* (▲) (*n* = 12), and *Trachurus trachurus* (□) (*n* = 13) for $\delta^{15}N$ (*a*) and $\delta^{13}C$ (*b*).



ecosystem of the Bay of Biscay, and by Savoye (2001) for the Bay of Brest. These similar values obtained in different ecosystems during different periods of the year support the notion that $\delta^{15}N$ values for POM remain quite stable throughout the year, particularly in offshore areas like the Great Mud Bank, which are subjected to fewer terrestrial inputs than coastal areas. Consequently the difference in the sampling periods for POM and fauna should not induce error in interpreting the organization of the trophic system. Goericke et al. (1994) showed that the $\delta^{13}C$ values for POM in surface waters of temperate marine ecosystems (between latitudes 45° and 50°N) range between −25‰ and −18‰. On the continental shelf of the Bay of Biscay, Fontugne and Jouanneau (1987) measured $\delta^{13}C$ values for POM ranging between –24‰ and –21‰, close to our results. The absence of a significant difference between $\delta^{13}C$ values for surface-layer and bottom POM further indicates that sedimentation occurs without qualitative transformation.

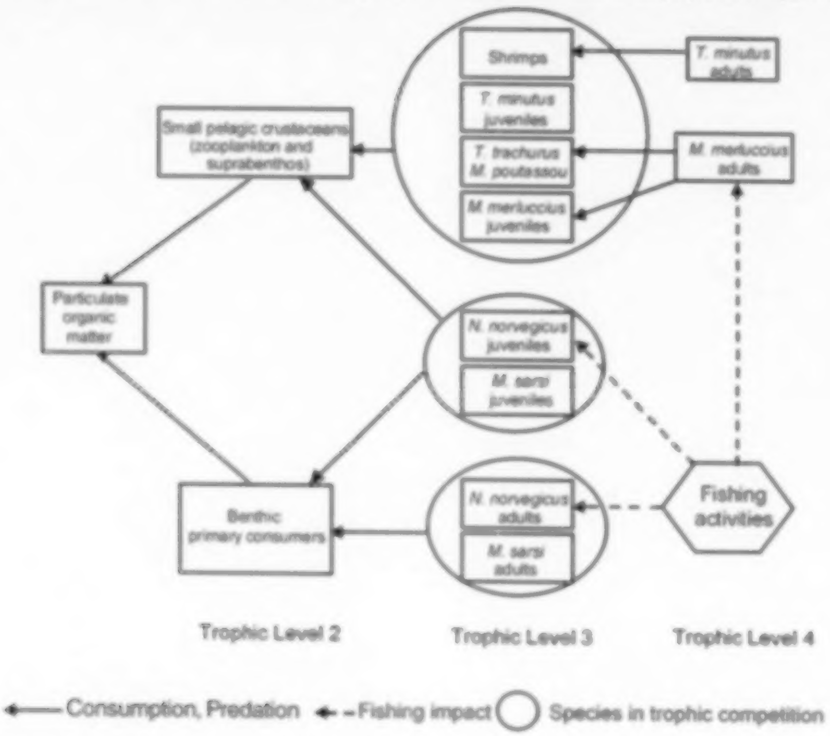
A difference of 3.4‰ in $\delta^{15}N$ values is observed between ratios for POM and those for ascidians and the crustacean *C. subterranea*, two taxa regarded as suspension feeders exploiting the water layer above the sediment (Fénaux 1966; Nickell and Atkinson 1995). Such a difference is also found for zooplankton and suprabenthos, which are known to feed on POM in the water column. These findings validate the value for the enrichment factor retained in the equation used to determine trophic level and confirm that these taxa are primary consumers. The deposit feeders *N. sulcata* (Dame 1996) and *Alpheus glaber* (Froglia et al. 1996) are both enriched in ^{15}N, in contrast to suspension feeders, revealing an intermediate trophic level (2.5). Owens (1987) found that the $\delta^{15}N$ values of plant species increased throughout the decomposition process, and if one considers phytoplankton specifically, the amount of total N decreased in conjunction with the decomposition of cells, whereas $\delta^{15}N$ abundance increased gradually in residual organic matter (Wada and Hattori 1991). As no primary production can occur at a mean depth of 110 m in the Great Mud Bank ecosystem, the diet of deposit feeders must be composed of deposited material that is a mixture of detritic and recycled material from benthic organisms. This feeding pattern is quite different from that of coastal shallow ecosystems in which microphytobenthos can be an important food source for deposit feeders (Riera et al. 1996; Middelburg et al. 2000).

The ranges of differences in $\delta^{13}C$ values between POM and benthic primary consumers and between suspension and deposit feeders seem to be a general trend in coastal (Herman et al. 2000) as well as offshore (Hobson et al. 1995, 2002) benthic ecosystems. Herman et al. (2000), studying a small tidal flat, demonstrated such differences among benthic primary consumers, suspension feeders (mainly on a diet of planktonic algae) exhibiting $\delta^{13}C$ values 4‰–6‰ lower than those of deposit feeders (mainly on a diet of benthic microalgae). The trophic quality of the organic matter depends on the various stages of decomposition that it undergoes that are associated with bacteria (Langdon and Newell 1990) and Protozoa (Posh and Arndt 1996). It has been suggested, however, that deposited detritic phytoplankton could be enriched in ^{13}C in the bottom nepheloid layer as a result of recycling across the microbial loop (Goering et al. 1990; Hobson et al. 1995). The different feeding behaviors of the species may, however, also be a factor that accounts for the observed differences in $\delta^{13}C$ values, as some species are nonselective and others selective microphages. In the latter case, particle selection may be based on quality as well as size. Some bivalves have a diet based not only on deposited material, microheterotrophs, and bacteria (Kang et al. 1999; Kreeger and Newell 2001), but also on dissolved organic carbon (Roditi et al. 2000). Feeding preferences such as these could lead to variations in the $\delta^{13}C$ values of species considered to be broadly part of the deposit-feeder group. These results can be compared with those obtained by Fry (1988) for Georges Bank, an exploited benthic ecosystem in the same depth range. In that study, no marked variability of $\delta^{15}N$ values was found between benthic and pelagic primary consumers (copepods, Euphausiacea, bivalves, benthic amphipods), but $\delta^{13}C$ values for pelagic copepods and Euphausiacea were 2‰–3‰ lower than those for benthic primary consumers. It was concluded that these differences reflect the influence of two different food sources or a modification of food quality.

The bivalve *Aequipecten opercularis* is a filter-feeding species that feeds on organic particles in suspension in the first 2 cm of the water layer above the sediment surface. The position of the pectinid species in this "benthic deposit feeder group" indicates that suspension feeders must be carefully considered in energy budgets and functional analyses and that behavior and nutritional organs are characteristics that are not always sufficient to place species in specific trophic groups. Furthermore, we can deduce that in the ecosystem of the Great Mud Bank, the quality of the suspended particles changes drastically in the first centimetres of the water layer above the sediment and that this change is sufficiently stable in time to induce differences in isotopic signatures in the tissue of consumers and (or) that these species could differ in particle-selection efficiency. *Aequipecten opercularis* lies on the sediment and could possibly ingest a greater amount of superficial film at the interface than ascidians, whose inhalant siphons are a few centimetres above the interface. In the same manner, the polychaete *Hyalinoecia fauveli* is positioned in such a way that it feeds at the apex of its tube, erected 4 or 5 cm above the sediment.

The third trophic level of the food web is composed of benthic and pelagic Crustacea, Polychaeta, and benthopelagic fish ($\delta^{15}N$ averages 11‰). Among these species, analysis of the known behavioral features identified two groups: endo- and epi-benthic species and demersal and suprabenthic species. In general, the trophic positions predicted by $\delta^{15}N$ values were in good agreement with data in the literature concerning the diet and feeding behavior of fishes and invertebrates. However, three polychaete species that showed unexpected values require further investigation. *Notomastus latericeus*, with an extensive distribution in coastal and intertidal muddy sediments, is considered to be a subsurface deposit feeder, like most members of the family Capitellidae. *Terebellides stroemi*, a tubiculous member of the family Terebellidae, is regarded as a surface deposit feeder, catching particles of the organic superficial film with its palps and cirri (Fauchald and Jumars 1979). Our results, however, have shown great variations in the $\delta^{13}C$ values of these species, which suggests considerable variability of the deposit feeder's diet (Riera and Hubas 2003), whereas $\delta^{15}N$ values were higher than expected from its trophic behavior (i.e., primary consumer) as described in the literature. Another species, *Lumbrinereis impatiens*, is a small surface-dwelling carnivorous member of the Lumbrinereidae that supposedly feeds on benthic prey (Fauchald and Jumars 1979). Its $\delta^{13}C$ values were lower than expected, which explains why it was grouped with the cluster of "pelagic-component" species. Further isotopic measurements are required over a large environmental gradient to confirm these values. It is likely, however, that conclusions about the feeding behavior of these species are based on a selection of ingested foods mainly composed of animal detritus and (or) micro- and meio-fauna. As previously noted for the bivalve *A. opercularis*, these results emphasize another advantage of stable-isotope measurement: the identification of species

Fig. 7. Trophic relationships of *Merluccius merluccius* and *Nephrops norvegicus* and their main prey and competitors.


Shrimps
T. minutus adults
T. minutus juveniles
Small pelagic crustaceans (zooplankton and suprabenthos)
T. trachurus
M. poutassou
M. merluccius adults
M. merluccius juveniles
Particulate organic matter
N. norvegicus juveniles
M. sarsi juveniles
Benthic primary consumers
N. norvegicus adults
M. sarsi adults
Fishing activities
Trophic Level 2
Trophic Level 3
Trophic Level 4
Consumption, Predation
Fishing impact
Species in trophic competition

whose feeding habits need further investigation. These species, which show no correlation between their feeding habits, as deduced from their morphology and behavior, and the carbon and nitrogen isotope values in their tissues should be reconsidered in different types of environmental situations in order to specify more accurately their position in the food web.

The diets of many species are modified throughout their life cycle, particularly as their increasing size facilitates adaptation to other prey. In some studies, stable carbon and nitrogen isotope ratios have been used as tracers of the diet changes of a species according to its life cycle and the variability of the ecosystem exploited for food. These studies have focused on fishes (Wainright et al. 1993), pelagic invertebrates (Riera et al. 2000), or benthic invertebrates (Kang et al. 1999). Our results indicate that juvenile *M. merluccius* may be considered third-level consumers and that their diet is mainly of pelagic origin. *Micromesistius poutassou* and *T. trachurus* exhibited very similar values to those of the juvenile hake for both stable isotopes (Kruskal–Wallis *H* test), indicating closely related diets. This finding is in agreement with the analyses of stomach contents, which proved that the prey of those species, for the size class considered here, are mainly pelagic crustaceans (copepods and Euphausiacea; Murta et al. 1993; Guichet 1995; Sorbe 1980). A shift in the hake's diet is revealed by large differences in stable-isotope values for adult *M. merluccius* (39–47 cm) compared with their juveniles (2.8‰ and 1.8‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively). *Micromesistius poutassou* and *T. trachurus* are the main prey of these adults (together with *Engraulis encrasicolus*; Guichet 1995).

The 2‰ enrichment of $\delta^{15}N$ and the stable $\delta^{13}C$ values for *T. minutus* indicate changes in diet without any major changes in food origin. Armstrong (1982), who studied the stomach contents of this species, found that prey size increased with fish size, but that prey consisted of pelagic shrimps for all size classes. Riera et al. (2000) showed that changes in the diet of shrimps (fish prey) throughout their life cycle are correlated with changes in their $\delta^{15}N$ signature. Our results confirm this observation, i.e., *T. minutus* feed on the same kind of prey, but from different size classes, which induces changes in their isotopic signature throughout their lives.

The two benthic crustaceans, *N. norvegicus* and *M. sarsi*, showed the same pattern in terms of the evolution of stable carbon and nitrogen isotope ratios with size, therefore it can be deduced that both species feed on the same prey. Both species remain at the third trophic level (secondary consumers) throughout their benthic life, but the isotope values of carbon indicate that changes in diet occur as they increase in size. The $\delta^{13}C$ values (−18‰ to −17‰) of juveniles suggest a mixed diet of pelagic and benthic prey, whereas a strictly benthic diet is likely during the adult period. These results confirm that *N. norvegicus* is an opportunistic carnivore that exploits both benthic (mollusks and polychaetes) and bathypelagic (decapod larvae, Euphausiacea, copepods) prey (Lagardère 1977). These findings are in agreement with those obtained by Jennings et al. (2002) in the Celtic Sea, hence it can be deduced that the diet of *N. norvegicus* is quite homogeneous over a large geographical area. Unfortunately, differences in individual sizes and individual weights in the two studies limit comparisons. *Munida sarsi* and *N. norvegicus*, by occupying the sediment, compete for space, and by feeding on the same resources, compete for food. It can be concluded that *N. norvegicus* and *M. sarsi* are competitors throughout their life cycle and occupy close trophic niches in the ecosystem. Selective fishing of one of these two "sister species" (*N. norvegicus*) could have serious medium-term consequences for the *N. norvegicus* populations. The hypothesis is that *N. norvegicus* populations could be progressively replaced by those of *M. sarsi*, which could prevent or limit reconstitution of *N. norvegicus* stocks either by natural recruitment or by fisheries management.

The main features of the trophic interactions in which *N. norvegicus* and *M. merluccius* are involved are summarized in Fig. 7. Predator–prey interactions explain the transition from one trophic level to another, and trophic competition explains the position of a species within a group. In the case of *N. norvegicus*, the consequences of overfishing are likely to be either an increase of *M. sarsi* biomass and (or) an increase of their prey.

A complex structure was identified within the trophic web: each of the three main taxonomic groups (crustaceans, polychaetes, fishes) had species at the different trophic levels, which suggests a high degree of specific and functional biodiversity. On a wider basis, this study brings to our attention the complex interactions between the target species and their prey and competitors and suggests that the whole trophic structure could be modified by exploitation of the dominant species' populations by modifying their size-class structure. This implies that in a fishery context, trophic interactions between target and nontarget species and their prey must be better understood if exploitation is to be optimized while conserving the biodiversity of this ecosystem. As the present study was mainly concerned with large epifauna and demersal species, further investigations need to focus on the sedimentary compartment, including the macrofauna, meiofauna, and bacteria, which are the bases of the benthic food-web component.

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Rapid shift in zooplankton community composition on the northeast Pacific shelf during the 1998–1999 El Niño – La Niña event

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Abstract: The 1997–1998 El Niño was one of the strongest ocean warming events in the historical record followed by an equally strong cold La Niña event in 1999. We observed a rapid shift in the marine zooplankton assemblage found in the transitional area between the California Current domain and the Alaska Gyre domain. Nonmetric multidimensional scaling revealed that the shift in species composition was caused primarily by changes in the relative abundance of subtropical neritic copepods normally found in the California Current domain. In 1998, the subtropical neritic copepods *Paracalanus*, *Ctenocalanus*, and *Corycaeus* were found as far north as 56°N and occurred in 100%, 96%, and 51% of 1998 samples versus 16%, 5%, and 3% of 1999 samples. The type and magnitude of change were similar to those observed off central Oregon but differed from those observed in southeast Alaska. Results support the hypothesis that anomalous poleward transport can inject significant California Current water into the coastal circulation of the Alaska Gyre and suggest that alongshore connectivity between the two domains may extend farther to the north than previously thought.

Résumé : L'El Niño de 1997–1998 a été l'un des épisodes de réchauffement de l'océan les plus intenses de l'histoire, suivi en 1999 par un épisode de refroidissement La Niña également important. Il s'est produit un changement rapide de la communauté de zooplancton marin dans la zone de transition entre le domaine du courant de Californie et celui du tourbillon d'Alaska. Un cadrage multidimensionnel non métrique montre que le changement de composition spécifique est causé principalement par des modifications de l'abondance relative de copépodes néritiques subtropicaux trouvés normalement dans le domaine du courant de Californie. En 1998, les copépodes néritiques subtropicaux *Paracalanus*, *Ctenocalanus* et *Corycaeus* se retrouvaient au nord jusqu'à 56 °N et ils étaient présents dans 100, 96 et 51 % des prélèvements, alors qu'en 1999, ils apparaissaient dans 16, 5 et 3 % des prélèvements. Le type et l'importance des changements ressemblaient à ceux observés au large du centre de l'Oregon, mais ils étaient différents de ceux observés dans le sud-est de l'Alaska. Ces résultats s'accordent avec l'hypothèse selon laquelle un transport inusité vers le pôle peut injecter des quantités significatives d'eau du courant de Californie dans le système de circulation côtière du tourbillon de l'Alaska; ils indiquent aussi que la connectivité des deux domaines le long de la côte peut s'étendre plus au nord que l'on ne le croyait antérieurement.

[Traduit par la Rédaction]

Introduction

El Niño – La Niña events are known to cause dramatic shifts in ocean water properties, food web structure, species distributions, and fisheries production in the eastern subarctic Pacific (e.g., Wooster and Fluharty 1985; Chavez et al. 2002). The 1997–1998 El Niño was one of the largest ocean perturbations in the historical record: beginning in February 1998, ocean temperatures in the upper 200 m of the coastal northeast Pacific were 2–3 °C warmer than climatological averages (Castro et al. 2002). It was followed in 1999 by an extremely strong La Niña. The rapid shift from a warm-ocean to a cool-ocean condition was accompanied by equally rapid shifts in plankton assemblages in the Oregon coast upwelling ecosystem (Peterson and Keister 2002; Peterson et al. 2002) and the upwelling ecosystem of southwestern British Columbia (Mackas et al. 2001; Mackas and Galbraith 2002). In contrast, no strong shifts in plankton species composition were detected in the Alaska downwelling ecosystem west of Prince William Sound (Coyle and Pinchuk 2003). Very little is known about El Niño impacts on plankton assemblages at the interface between the California and Alaska domains on the northeast Pacific continental shelf in the transition from the southward-flowing, upwelling California Current domain to the northward-flowing, downwelling Alaska Gyre domain (Thomson 1981; Ware and McFarlane 1989). This is due to sparse data from the central and northern coasts of British Columbia, both in general and for El Niño – La Niña events in particular (Perry and Waddell 1997).

During 1998 and 1999, we collected zooplankton as a part of surveys of the central and northern British Columbia and

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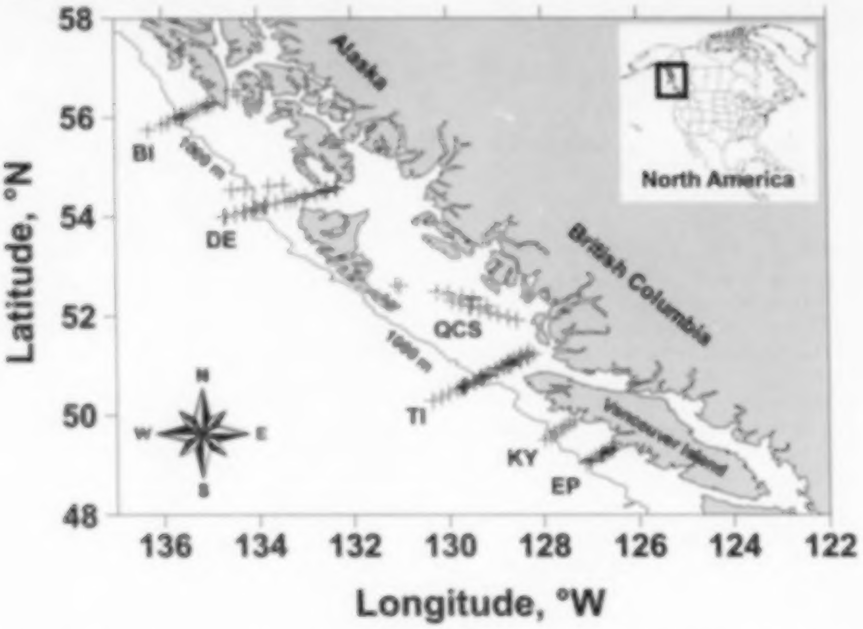
Fig. 1. Location of zooplankton net tows. Transect abbreviations: BI, Baranof Island; DE, Dixon Entrance; QCS, Queen Charlotte Sound; TI, Triangle Island; KY, Kyuquot Sound; EP, Estevan Point.



southeast Alaska coasts, an area that had not been sampled during the 1982–1983 El Niño event. The objectives of our analyses are to (*i*) describe how zooplankton community composition changed between 1998 and 1999 in the transitional domain between the California Current and Alaska Gyre ecosystems, (*ii*) determine which taxa were responsible for major changes in community composition, and (*iii*) establish the northward extent of changes in zooplankton composition seen within the survey area. Particular attention is paid to copepod genera that have been used as indicators of warmwater communities (Peterson and Miller 1975), including genera from subtropical shelf communities associated with poleward transport of California Current waters (*Paracalanus*, *Ctenocalanus*, and *Corycaeus*) and genera from subtropical offshore communities associated with onshore transport of subtropical gyre waters (*Mesocalanus* and *Clausocalanus*). This study provides a connection and comparison with contemporary work in Oregon (Peterson and Keister 2002; Peterson et al. 2002), southern British Columbia (Mackas et al. 2001; Mackas and Galbraith 2002), and Alaska (Cooney et al. 2001; Coyle and Pinchuk 2003).

Materials and methods

Sample collection

Zooplankton samples were analyzed from the Canadian Coast Guard Ship *W.E. Ricker* during seven research cruises: June, July, August, and October 1998 and May, June, and October 1999. Six cross-shelf transects between 49°N and 56°N were sampled, although not all transects or stations were sampled on all cruises (Fig. 1; Table 1). Because only temperature and salinity were available as water mass tracers, we were unable to reliably resolve water mass identification with physical data available to us in this dynamic and complex area (Mackas et al. 1987). For our purposes, we classified a sample as being from the continental shelf if that sample came from water shallower than 200 m bottom depth. In the cases of Dixon Entrance, Queen Charlotte Sound, and Triangle Island, where deeper trenches cut into the main contour of the shelf, we included the inshore-most six and seven stations of Dixon Entrance and Triangle Island and all of the Queen Charlotte Sound stations as shelf stations. Although our analysis will focus on 82 spring–summer shelf samples (May, June, July, and August), additional data from 53 spring–summer samples off the shelf were taken during the same cruises, and these were used to provide additional distributional information for warmwater indicator taxa.

Zooplankton were captured with a 0.58-m-diameter, 253-μm black mesh bongo net hauled obliquely from the stern while the ship was under way at a speed of approximately 1–1.5 m·s^{-1} (2–3 kn). We measured the wire angle of the net and the length of wire deployed to calculate the maximum depth reached by the net. Volume filtered per tow was measured with a calibrated General Oceanics flowmeter mounted inside the mouth of one of the nets on the bongo frame. The net sampled the upper 150 m of the water column, or from within approximately 10 m of the bottom to the surface in water depths shallower than 150 m. Zooplankton from the net without the flowmeter were sorted into four size fractions by gently rinsing the sample through four sieves of progressively smaller mesh sizes (8-, 1.7-, 1.0-, and 0.25-mm mesh). Each size fraction was frozen at sea for transport back to the laboratory, dried in an oven for 6 days at 52 °C, and then weighed to the nearest milligram.

Zooplankton from the net containing the flowmeter were preserved in 10% formalin and seawater solution buffered

Table 1. Distribution of zooplankton samples by transect, year, and month.

Transect and latitude		June 1998	July 1998	Aug. 1998	Oct. 1998	May 1999	June 1999	Oct. 1999	Total
BI, 56°N	Shelf	—	3 (2)	2 (0)	2 (0)	3	—	—	10
	Offshore	—	7	—	6 (0)	7	—	2 (0)	22
DE, 54°N	Shelf	6 (4)	6	4	5 (0)	6	6	2	35
	Offshore	5	6	—	5 (0)	2	4	5 (2)	27
QCS, 52°N	Shelf	—	8 (7)	7 (6)	—	—	—	4	19
TI, 51°N	Shelf	4	5	7	6	7	7 (6)	—	36
	Offshore	1	5	3	1	6	4	—	20
KY, 49.7°N	Shelf	—	—	—	—	—	—	3	3
	Offshore	—	—	—	—	—	—	4	4
EP, 49.5°N	Shelf	—	—	—	—	6 (5)	4	4	14
	Offshore	—	—	—	—	1	2	1	4
Total		16	40	23	25	38	27	25	194

Note: Where preserved samples were lost or damaged but a frozen sample was retained for dry weight measures, parentheses indicate the number of preserved samples available for taxonomic analysis. A dash indicates that no samples were available. BI, Baranof Island; DE, Dixon Entrance; QCS, Queen Charlotte Sound; TI, Triangle Island; KY, Kyuquot Sound; EP, Estevan Point.

with sodium borate. Whole, preserved samples were split to generate subsamples with 30–50 individuals per major taxon and a within-subsample counting error of ±25%–40% (Mackas et al. 1991; Mackas 1992). Plankton from each subsample were sorted and counted. For each taxon, numerical abundance per cubic metre was extrapolated to the whole sample by adjusting the subsample volume split for that taxon to the total volume sampled by the tow.

Distribution and abundance of warmwater indicators

Both presence–absence and abundance data for *Paracalanus*, *Ctenocalanus*, *Mesocalanus*, and *Clausocalanus* were plotted against latitude to determine how far north each taxon was detected in our surveys.

To check the validity of assuming that *Paracalanus* and *Ctenocalanus* are indicators of primarily shelf water and *Mesocalanus* and *Clausocalanus* are indicators of primarily offshore water, the presence–absence of each genus in shelf versus offshore samples was compared with the null hypothesis that all genera would occur equally in shelf and offshore samples. We used a goodness-of-fit test to compare observed versus expected occurrences in all available tows from 1998 and 1999, including October tows (163 samples total).

Nonmetric multidimensional scaling (NMDS) ordination of community change

We used NMDS, a nonparametric ordination technique, to compare shelf zooplankton community composition between 1998 and 1999 (Clarke and Warwick 1994; Legendre and Legendre 1998). Ordination analyses were performed on relative numerical abundances in a two-step process. First, the samples-by-taxa matrix of $\log_{10}(x + 1)$-transformed density data was used to generate Bray–Curtis similarity and dissimilarity matrices for all possible pairwise comparisons of samples (Bray and Curtis 1957). Second, the similarity matrix was then subjected to the NMDS ordination procedure to visualize the similarities among samples. The details of the similarity and ordination calculations are explained below.

Densities were $\log_{10}(x + 1)$ transformed before computation of the Bray–Curtis similarity coefficients to allow the similarity coefficient to reflect changes in both numerically dominant taxa and relatively uncommon taxa, which might be important indicators. Without such a transformation, the similarity coefficient is insensitive to the appearance of uncommon or rare species in the community and oversensitive to absolute abundances (Legendre and Legendre 1998). For a set of p species in n samples, the Bray–Curtis similarity S_{jk} coefficient (Bray and Curtis 1957) defines the similarity between the jth and kth pair of samples as

$$(1) \qquad S_{jk} = 100 \times \left[1 - \frac{\sum_{i=1}^{p} \left| y_{ij} - y_{ik} \right|}{\sum_{i=1}^{p} (y_{ij} + y_{ik})} \right]$$

where y_{ij} and y_{ik} are the transformed abundances of species i in samples j and k, respectively. A value of 100 indicates that samples are identical in species composition and relative abundance; a value of 0 indicates that there are no common species between two samples. The Bray–Curtis dissimilarity coefficient between the jth and kth pair of samples is defined as

$$(2) \qquad D_{jk} = 100 - S_{jk}$$

Changes in spring–summer zooplankton composition on the continental shelf were examined with NMDS plots derived from the Bray–Curtis similarity matrices. Matrices were analyzed and plot coordinates generated with SYSTAT 10 (SPSS, Inc. 2000). The ordination procedure reduces the dimensionality of pairwise data relationships by representing each sample as a point in two- or three-dimensional space such that the NMDS distances between any two points deviate as little as possible from the rank ordering of Bray–Curtis similarities calculated from pairwise comparisons of the samples. This visualization technique seeks to preserve rank order of sample similarities, not necessarily the absolute distances among similarities. Samples close to each other on the NMDS plot are similar in community composition, and samples that are farther apart on the NMDS plot are dissimilar in community composition. For more detailed discussion of the NMDS procedure in ecological contexts, see

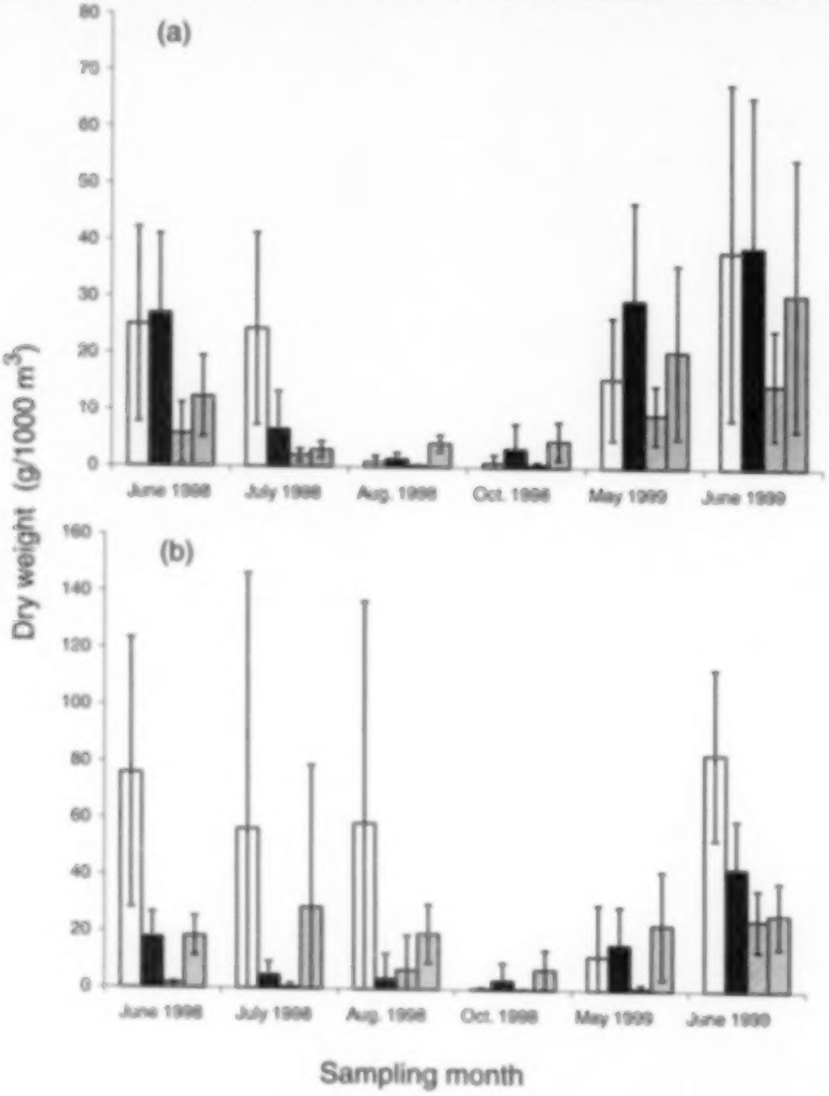


Fig. 2. Mean and standard deviation of dry zooplankton biomass by size fraction (open bars, >8.0 mm; solid bars, 1.7–8 mm; hatched bars, 1.0–1.7 mm; shaded bars, 0.25–1.0 mm). (*a*) Dixon Entrance transect at 54°N; (*b*) Triangle Island transect at 51°N.

Clarke and Warwick (1994) and Legendre and Legendre (1998). To avoid NMDS solutions that misrepresented sample relationships by converging to local minima in the two- and three-dimensional plots, the NMDS was performed sequentially on four-, three-, and two-dimensional configurations derived from an initial five-dimensional analysis with a stress <0.1 (Legendre and Legendre 1998, p. 446).

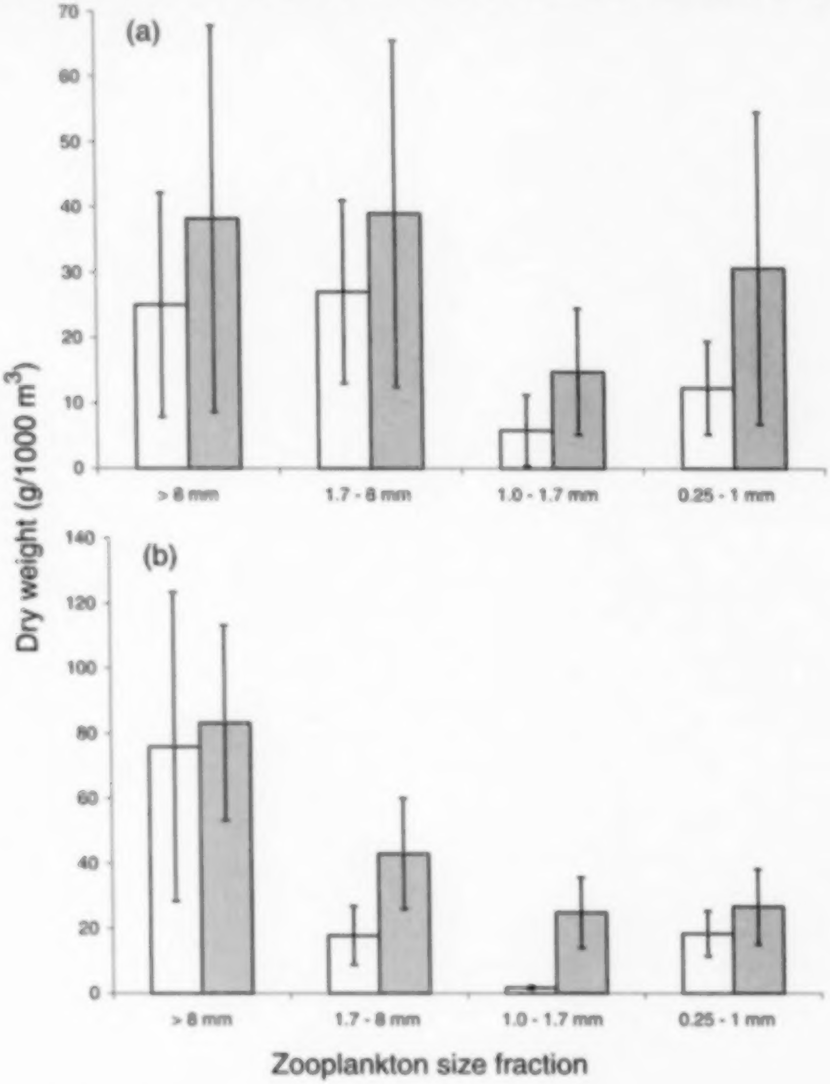
To determine which species contributed the most to the dissimilarity between 1998 and 1999 community compositions, a decomposition of individual species' contributions to the average Bray–Curtis dissimilarity between years was performed (Clarke and Warwick 1994). To better isolate the 1998 versus 1999 comparison from within-year seasonal changes in community composition, only samples from transects that were sampled in June of both years were included in the decomposition analysis. These transects were Triangle Island (51°N) and Dixon Entrance (54°N).

For *n* samples in 1998 and *m* samples in 1999, the average dissimilarity between 1998 and 1999 was defined as

$$(3)\qquad \bar{\bar{\delta}} = \frac{\sum_{j=1}^{n}\sum_{k=1}^{m} D_{jk}}{nm}$$

The average contribution of species *i* to the average dissimilarity between 1998 and 1999 was defined as

Fig. 3. Mean and standard deviation of dry zooplankton biomass by size fraction and region, June 1998 (open bars) versus June 1999 (shaded bars). (*a*) Dixon Entrance transect at 54°N; (*b*) Triangle Island transect at 51°N. Note the different scale of the *y* axis on each panel.



$$\bar{\delta}(i) = \frac{\sum_{j=1}^{n}\sum_{k=1}^{m}\delta_{jk}(i)}{nm} \tag{4}$$

where $\delta_{jk}(i)$ is the *i*th term of eq. 2.

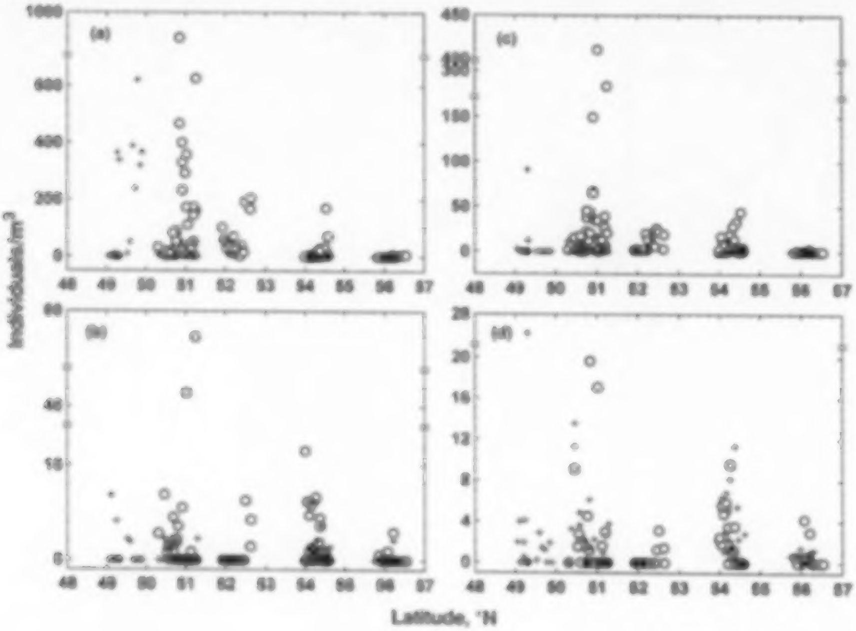
Results

Distribution and abundance of warmwater indicators

Bulk measures of shelf zooplankton biomass in our data showed highest abundance and biomass during May, June, and July samples, with reduced biomass in October (Fig. 2). The range of variation in zooplankton biomass at 51°N was almost twice that of 54°N (0.1–83.3 versus 0.3–38.2 g dry weight·1000 m^{-3}). Mean shelf biomass in June was lower in 1998 than in 1999 for all size-classes at both 51°N and 54°N (Fig. 3). However, mean biomass was statistically different for only two size-classes at 51°N (t test on $\log_{10}(x + 1)$-transformed data: 1.7–8 mm, $P = 0.01$; 1.0–1.7 mm, $P < 0.001$; all others not significant, $P > 0.05$).

Paracalanus and *Ctenocalanus*, normally associated with warmer shelf waters in the California Current system, occurred in 100% and 96% of 1998 spring–summer shelf samples (June, July, and August) but only 16% and 5% of 1999 spring–summer shelf samples (May and June), respectively. These taxa were present from 49°N to 56°N in 1998 but were most abundant south of 52°N (Fig. 4). In June, *Para-*

Fig. 4. Copepod abundance versus latitude and year for warmwater genera, all available net tows. (*a*) *Paracalanus*; (*b*) *Clausocalanus*; (*c*) *Ctenocalanus*; (*d*) *Mesocalanus*. Open circles indicate samples from 1998 and crosses indicate samples from 1999. Note the different scale break on the *y* axis of each panel.



calanus and *Ctenocalanus* accounted for 11%–18% of relative zooplankton abundance in 1998 but less than 1% of relative abundance in 1999 (Fig. 5). These two taxa accounted for a larger proportion of community membership at 51°N than at 54°N (18% versus 11%). Mean shelf densities of *Ctenocalanus* declined in abundance between June and July 1998, but *Paracalanus* remained in high densities throughout the summer and early fall of 1998 (Table 2). In May and June of 1999, *Paracalanus* and *Ctenocalanus* were effectively absent from the plankton when compared with June 1998. However, *Paracalanus* reappeared on the shelf in early October 1999 at densities comparable with those of 1998. Another warmwater neritic copepod, *Corycaeus* (Peterson et al. 2002), occurred at 51% of all 1998 stations and was relatively abundant in June of 1998 at 51°N (Fig. 5), but this genus was only detected at one station in 1999.

On the shelf, *Clausocalanus* and *Mesocalanus* were present in 33% and 36% of 1998 samples compared with 11% and 43% of 1999 samples, respectively. These taxa also occurred throughout the study area between 49°N and 56°N. *Clausocalanus* and *Mesocalanus* accounted for less than 1% of relative abundance in both years. *Clausocalanus* and *Mesocalanus* occurred in low densities during both 1998 and 1999 ($<10\cdot m^{-3}$, except for *Clausocalanus* near Triangle Island in June 1998, where it was found in densities of $26.1\cdot m^{-3}$).

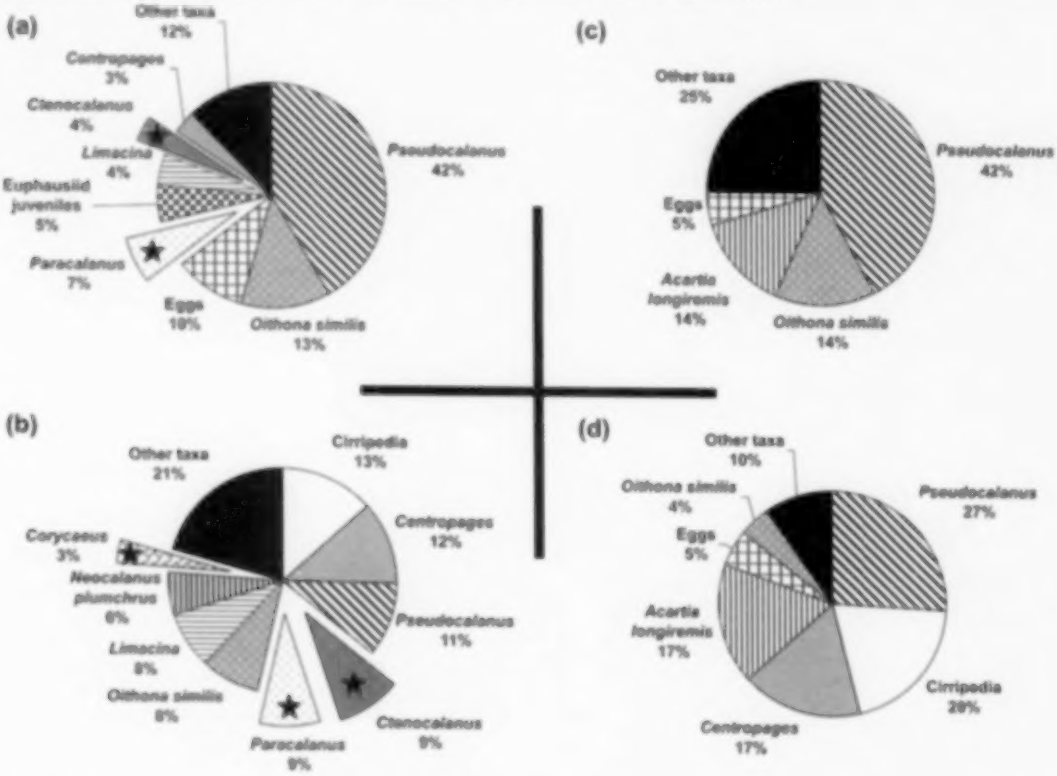
Analysis of presence–absence data indicated that *Paracalanus* was significantly associated with shelf samples, whereas *Clausocalanus* and *Mesocalanus* were significantly associated with offshore samples (Table 3). In our data, *Ctenocalanus* did not exhibit a significant association with either shelf or offshore samples.

NMDS ordination of community change

In the three-dimensional NMDS representation, samples from 1998 and 1999 tended to group separately (Kruskal type 1 stress = 0.129) (Fig. 6), indicating that shelf community species composition did differ significantly between 1998 and 1999. A two-dimensional NMDS representation showed an even clearer separation between 1998 and 1999 samples in dimension 2, although this configuration had a somewhat greater degree of stress (Kruskal type 1 stress = 0.199) (Fig. 7). The two-dimensional plot was useful for examining the relationships among shelf regions. Samples from 51°N tended to fall on the left side of the plot (triangles, negative dimension 1 values), while samples from 52°N, 54°N, and 56°N fell on the right side of the plot (squares and circles, positive dimension 1 values). This would indicate that the magnitude or type of community change north of 51°N was somewhat distinct from that of communities near 51°N.

Mean Bray–Curtis dissimilarities from 51°N were significantly larger than those from 54°N ($n = 24$ for each region; $\bar{\delta} = 56.5$ versus 41.9, t test for unequal variances, 41 df, $P < 0.0001$) and had significantly higher variance at 51°N (97.5 versus 48.1, F test, 23 df, $P = 0.05$). This result showed that the magnitude of change between 1998 and 1999 at 51°N was larger than at 54°N and explained the separation between these regions in the two-dimensional NMDS plot. However, data from the dissimilarity decomposition indicate that communities from 51°N and 54°N underwent similar

Fig. 5. Relative numerical composition of zooplankton. The relative abundance was calculated from the sum of all individuals over all shelf tows. Only taxa that accounted for more than 3% of relative abundance are shown as separate slices in the pie. Stars indicate taxa with warmwater affinities. (*a*) Dixon Entrance transect at 54°N, June 1998; (*b*) Triangle Island transect at 51°N, June 1998; (*c*) Dixon Entrance transect at 54°N, June 1999; (*d*) Triangle Island transect at 51°N, June 1999.



compositional changes between 1998 and 1999. From the total of 64 taxonomic categories in our analysis, only 18 taxa contributed more than 3% to between-year sample dissimilarities (Tables 4 and 5). Changes in these taxa accounted for 69.5% and 50.5% of the total differences at 51°N and 54°N, respectively. For both 51°N and 54°N, the largest contributions to between-year differences came from *Paracalanus* and *Ctenocalanus*, the two warmwater genera normally abundant in subtropical neritic water from the California Current system (Mackas and Galbraith 2002; Peterson et al. 2002). These genera accounted for between 6.0% and 7.4% of mean dissimilarity. Five other taxonomic categories contributing to community changes were shared between regions: *Centropages*, Cirripedia (barnacle larvae), eggs, euphausiid juveniles, and *Limacina* spp. (pteropods).

Measurable contributions to community change also came from other taxa, although these contributions were not shared between sample regions. At 51°N only, the subarctic oceanic copepod *Neocalanus plumchrus* accounted for 6.4% of community differences, and the subtropical neritic copepod *Corycaeus anglicus* contributed 5.8% to community differences. *Neocalanus plumchrus* was more abundant in our June 1998 shelf samples than in those from June 1999 (mean abundance 145 versus 0.3 individuals·m^{-3}, respectively), as was *C. anglicus* (82 versus 0 individuals·m^{-3}). At 54°N only, the subarctic neritic copepod *Acartia longiremis* accounted for 6.7% of community differences. This species was less abundant in 1998 than in 1999 (3.2 versus 104 individuals·m^{-3}, respectively).

Discussion

In the transitional study region, shelf zooplankton biomass trends were quantitatively similar to that described for the southwestern Vancouver Island shelf (Mackas 1992): high abundance and biomass observed in May–June–July followed by significant reductions in biomass during October. Zooplankton community composition was also similar to that previously described for the southwestern Vancouver Island shelf, with smaller copepods (<2 mm) such as *Pseudocalanus*, *Oithona*, *Centropages*, and *Acartia* being numerical dominants. Raw data, NMDS analysis, and dissimilarity decomposition all show evidence of a relatively rapid plankton community shift between 1998 and 1999. This shift from an assemblage including significant numbers of *Paracalanus*, *Ctenocalanus*, and other warmwater taxa to a virtual absence of warmwater taxa was almost identical to the shifts reported from southern Vancouver Island (48°N–49°N) by Mackas

Table 2. Mean shelf abundances of four warmwater copepod genera.

Transect and latitude	Genus	June 1998	July 1998	Aug. 1998	Oct. 1998	May 1999	June 1999	Oct. 1999
BI, 56°N	*Paracalanus*	—	5.8	—	—	0	—	—
	Ctenocalanus	—	1.5	—	—	0	—	—
	Clausocalanus	—	1.5	—	—	0.7	—	—
	Mesocalanus	—	1.5	—	—	0.8	—	—
DE, 54°N	*Paracalanus*	49.8	20.7	17.1	—	0.1	0	2.0
	Ctenocalanus	28.7	5.5	3.8	—	0	0.8	0
	Clausocalanus	0.7	0.8	1.1	—	0.6	0	0
	Mesocalanus	0.3	0.7	0.2	—	2.0	1.5	1.4
QCS, 52°N	*Paracalanus*	—	91.1	54.5	—	—	—	25.7
	Ctenocalanus	—	12.1	7.0	—	—	—	3.9
	Clausocalanus	—	1.7	0	—	—	—	0
	Mesocalanus	—	0.8	0.5	—	—	—	0
TI, 51°N	*Paracalanus*	201.5	82.8	344.9	182.5	3.4	0.1	—
	Ctenocalanus	217.2	14.2	26.5	26.9	0	0	—
	Clausocalanus	26.1	0.7	0	0.1	0	0.4	—
	Mesocalanus	4.3	0.6	0.2	3.8	2.2	0	—
KY, 49.7°N	*Paracalanus*	—	—	—	—	—	—	493.5
	Ctenocalanus	—	—	—	—	—	—	0
	Clausocalanus	—	—	—	—	—	—	0
	Mesocalanus	—	—	—	—	—	—	0.7
EP, 49.2°N	*Paracalanus*	—	—	—	—	0	0.9	178.7
	Ctenocalanus	—	—	—	—	18.2	0	3.1
	Clausocalanus	—	—	—	—	0.8	0	0
	Mesocalanus	—	—	—	—	6.4	0.1	0

Note: Means were calculated from the sample sizes given in Table 1 and are reported as numbers of individuals per cubic metre. A dash indicates that no samples were available. BI, Baranof Island; DE, Dixon Entrance; QCS, Queen Charlotte Sound; TI, Triangle Island; KY, Kyuquot Sound; EP, Estevan Point.

Table 3. Goodness-of-fit analysis of presence–absence data for warmwater genera, shelf versus offshore samples.

	Observed frequencies				Expected frequencies
	Paracalanus	*Ctenocalanus*	*Clausocalanus*	*Mesocalanus*	
Shelf, present	69	53	22	31	51
Shelf, absent	33	49	80	71	51
Offshore, present	34	33	29	47	30.5
Offshore, absent	27	28	32	14	30.5
χ^2	13.5	0.57	33.1	33.5	
df	3	3	3	3	
P	0.004	0.90	<0.001	<0.001	

Note: The *P* values indicate the probability that the observed frequency differed from the expected frequency owing to purely random variation in the presence–absence distribution.

and Galbraith (2002) and Mackas et al. (2001) and from central to southern Oregon (43°N–45°N) by Peterson and Keister (2002) and Peterson et al. (2002). *Paracalanus* and *Ctenocalanus* abundance changes from 1998 to 1999 at 51°N were similar in magnitude to those reported from central Oregon at 44.7°N: a decrease from hundreds per cubic metre to 0–20·m^{-3} (Peterson et al. 2002). The disappearance of *C. anglicus* in 1999 was also similar between Oregon and our study. Given that summer gradients in derived upwelling and other transport indices decrease from Oregon (45°N) to central British Columbia (51°N) to northern British Columbia (54°N), and that the oceanographic and biogeographic boundaries between California Current type and Alaska-type communities have been generally been placed between 50°N and 51°N (Fulton and LeBrasseur 1985; Ware and McFarlane 1989), it is somewhat surprising that both the type and magnitude of community change at 51°N are so similar to coastal Oregon. We expected a weaker signal and less dramatic shift at this latitude and possibly no detectable shift at all from 54°N.

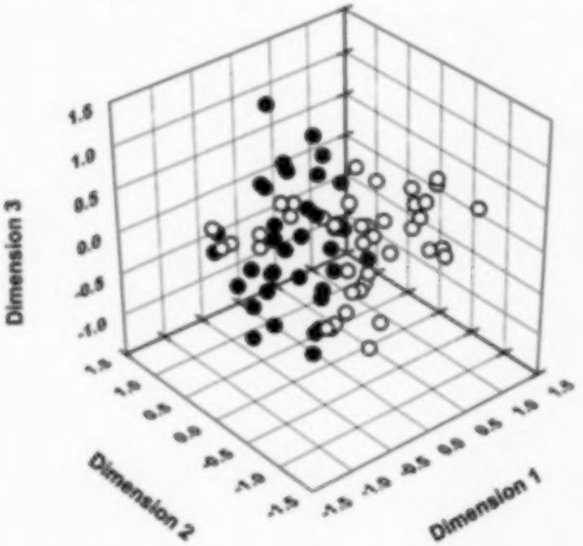
Our data support the Cooney and Coyle (1985) and Mackas et al. (2001) hypothesis that anomalous poleward transport can inject southern taxa into the coastal circulation of the Alaska Gyre. Zooplankton distributions suggest that alongshore connectivity between California Current shelf circulation and Gulf of Alaska shelf circulation reaches to at least 54°N and perhaps past 56°N. Warmwater taxa were detected on our northernmost line in southeastern Alaska (56°N). Results from the dissimilarity decomposition showed that warmwater species made significant contributions to com-

Table 4. Average contribution of the *i*th taxon, $\bar{\delta}(i)$, to the average dissimilarity ($\bar{\delta}$ = 41.9) between June 1998 and June 1999 community composition from Dixon Entrance samples, 54°N.

Taxon	$\bar{\delta}(i)$	SD	Contribution to $\bar{\delta}$ (%)	Cumulative contribution (%)
Ctenocalanus	3.07	1.07	7.3	7.3
Acartia longiremis	2.80	1.77	6.7	14.0
Paracalanus	2.51	1.49	6.0	20.0
Centropages	2.19	1.32	5.2	25.2
Euphausiid juveniles	1.97	1.26	4.7	29.9
Limacina	1.48	0.91	3.5	33.4
Eggs	1.41	1.21	3.4	36.8
Metridia pacifica	1.38	0.94	3.3	40.1
Cirripedia	1.37	1.05	3.3	43.4
Scolecithricella	1.36	0.83	3.2	46.6

Note: Only taxa that contributed >3% to the overall difference are shown.

Fig. 6. Three-dimensional, nonmetric multidimensional scaling plot of sample relationships, all summer shelf data, 1998 versus 1999. Open circles indicate samples from 1998 and solid circles indicate samples from 1999.



munity change to at least 54°N, although the magnitude of this change was less than seen at 51°N. Recent data from Cooney et al. (2001) and Coyle and Pinchuk (2003) indicated that warmwater offshore genera *Mesocalanus* and *Clausocalanus* are found inside and west of Prince William Sound, Alaska (60.5°N, 147°W), but adults of the shelf genus *Paracalanus* have been observed only once (Coyle and Pinchuk 2003), and adults of *Ctenocalanus* have not yet been reported. This suggests to us that alongshore shelf transport of warmwater taxa is very weak or entirely absent west of Prince William Sound. The exact transport mechanisms responsible for linking and changing plankton community structure in the California Current – Gulf of Alaska circulation cells still need to be investigated.

It is interesting that in our surveys, *Paracalanus* and *Ctenocalanus* were much more likely to be found in 1998 than in 1999, whereas *Clausocalanus* and *Mesocalanus* were found in similar frequencies and geographical ranges in 1998 and 1999. This may indicate that poleward, alongshore shelf transport of warm water in 1998 was greater than in 1999, while onshore transport of warm water was similar between years, although such a hypothesis remains to be tested. Alongshore transport measurements from the Oregon shelf (Kosro 2002) and the southern British Columbia shelf (Freeland and Thomson 1999) both show changes in poleward transport on the order of 10–20 cm·s^{-1}, or about 260–510 km·month^{-1}. The transport data are consistent with the hypothesis that changes in *Paracalanus* and perhaps *Ctenocalanus* abundances were driven by connectivity in alongshore shelf transport from Oregon to British Columbia. However, without greater temporal resolution in the zooplankton data, it is not possible to reconcile the appearance of warmwater taxa with specific transport mechanisms.

The observations that *Paracalanus* was also found near Estevan Point and in Queen Charlotte Sound during October 1999, a La Niña year, further support the idea that species assemblages are brought north during periods of strong poleward transport. In the case of October 1999, this may have been augmented by Vancouver Island Coastal Current transport. Northward transport of southern fauna is therefore not exclusively linked to El Niño anomalies, and there is the possibility that fall transport may in fact seed areas with populations that only express themselves under El Niño or other similarly warm conditions. Given that warmwater forms like *Mesocalanus* and *Paracalanus* make regular fall or winter appearances on the shelf in Oregon (Peterson et al. 2002), southern British Columbia (Mackas 1992), northern British Columbia – southeast Alaska (this study), and Prince William Sound, Alaska (Cooney et al. 2001), a study of the transition from summer to winter circulation patterns that followed the plankton community's response times could resolve the mechanisms through which community change is mediated.

Spring transitions may also be important agents of community change, both for zooplankton directly and for upper trophic levels that depend on plankton for food (e.g., Bertram et al. 2001). Of the groups contributing most to the average dissimilarity between years, several have strong cycles of growth or reproduction linked to surface water temperatures or the timing of the spring bloom. Mackas et al. (2001) used current shear measurements to estimate that the

Fig. 7. Two-dimensional nonmetric multidimensional scaling plot of sample relationships, all summer shelf data, 1998 (open symbols) versus 1999 (shaded symbols). BI, Baranof Island; DE, Dixon Entrance; QCS, Queen Charlotte Sound; TI, Triangle Island; KY, Kyuquot Sound; EP, Estevan Point.

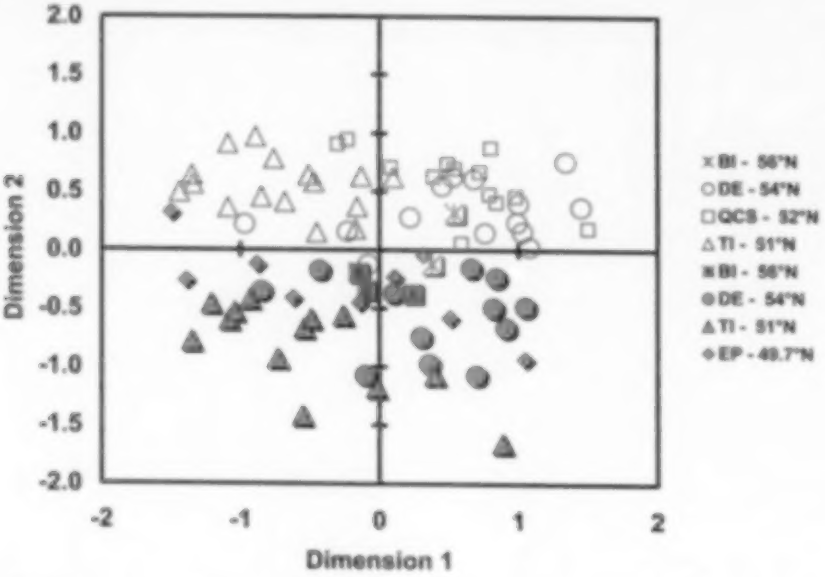


Table 5. Average contribution of the *i*th taxon, $\bar{\delta}(i)$, to the average dissimilarity ($\bar{\delta}$ = 56.5) between June 1998 and June 1999 community composition from Triangle Island samples, 51°N.

Taxon	$\bar{\delta}(i)$	SD	Contribution to $\bar{\delta}$ (%)	Cumulative contribution (%)
Paracalanus	4.18	0.35	7.4	7.4
Ctenocalanus	4.11	0.56	7.3	14.7
Neocalanus plumchrus	3.57	0.73	6.4	21.1
Corycaeus	3.27	0.88	5.8	26.9
Limacina	3.09	0.96	5.5	32.4
Carideans	2.83	0.38	5.0	37.4
Oithona similis	2.43	0.91	4.3	41.7
Cirripedia	2.40	1.54	4.3	46.0
Urochordate larvae	2.17	0.62	3.9	49.9
Euphausiid juveniles	2.06	1.04	3.7	53.6
Polycheates	1.96	0.82	3.5	57.1
Clausocalanus	1.82	1.25	3.2	60.3
Eggs	1.80	1.07	3.2	63.5
Calanus pacificus	1.73	1.03	3.1	66.6
Centropages	1.65	1.12	2.9	69.5

Note: Only taxa that contributed >3% to the overall difference are shown.

spring transition from winter to summer conditions off the southwest coast of Vancouver Island began on 25 February in 1998 compared with 14 April in 1999. This lends support to the hypothesis that spring bloom dynamics probably differed in the plankton community. The contributions of barnacle larvae, euphausiid juveniles, and eggs to community change suggest that some portion of change may have been caused by a difference in the spring transition periods for 1998 and 1999.

Our results for *N. plumchrus* surface abundance on the shelf appear to contradict what is known about that species life history, which is that biomass peaks in oceanic populations of *N. plumchrus* occur earlier in warm years than in cold years (Mackas et al. 1998), and therefore, one would have expected to see fewer *N. plumchrus* advected to surface shelf waters in 1998. However, our 1998 samples were taken on 2 and 3 June, whereas our 1999 samples were taken on 22 and 23 June. Given the likelihood of extreme patchiness of *N. plumchrus* on the shelf and that we sampled 20 days earlier in 1998 than in 1999, we believe that this result is likely to be a sampling artefact and not an indication that *N. plumchrus* biomass actually peaked earlier in 1999. The offshore samples from our surveys exhibit abundance patterns consistent with what has been observed in other studies, namely that offshore populations of *Neocalanus* peaked earlier in 1998 than in 1999 (Bertram et al. 2001).

Variation in ocean conditions and plankton community composition has important effects on feeding success and growth of salmon, other fishes, and seabirds (Perry et al. 1996; Bertram et al. 2001; Hedd et al. 2002). While changes in the abundance of warmwater taxa observed here are unlikely to have direct effects on upper trophic levels, they do indicate that changes in the oceanographic and trophic environment have taken place. Although seasonal variation and interannual variation of zooplankton standing stocks are relatively well characterized for southwestern Vancouver Island (48°N–49°N; see Mackas et al. (2001) for a recent review), similar information from the central and northern British Columbia coasts has been extremely limited (Perry and Waddell 1997). Given the importance of plankton as food for keystone forage fishes and early life history phases of commercially important fishes (Ware and McFarlane 1989), continued work on zooplankton dynamics in central and northern British Columbia is desirable. For example, biweekly or monthly sampling during August, September, and October could help resolve the question of whether fall transition winds, which increase poleward transport, are responsible for bringing *Paracalanus* populations and other warmwater taxa to central and northern British Columbia, even in the absence of El Niño phenomena. Such transport events may set the stage for growth of these populations during the El Niño. Results from this study suggest that poleward transport mechanisms play an important role in zooplankton community dynamics of this part of the British Columbia coast. Establishing the transport mechanisms, the source–sink dynamics for zooplankton assemblages, and the degree of connectivity between British Columbia, Alaska, and the California Current will be necessary to understand how El Niño and other climate changes impact ecologically and economically important coastal food webs.

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Energetic consequences of diet shifts in Lake Erie rainbow smelt (*Osmerus mordax*)

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Abstract: The diet of eastern Lake Erie rainbow smelt (*Osmerus mordax*) has changed since the 1960s, reflecting food resource shifts due to phosphorus reductions and the invasion of exotic species (dreissenid mussels and *Bythotrephes longimanus*). Since rainbow smelt growth was lower in the 1990s than in the 1960s, we considered diet changes as an explanation. A decrease in the proportion of zooplankton occurred in the summer (May–August) diet of two size classes (63–88 and 89–114 mm fork length) between 1961 and 1999. Within the zooplankton, the proportion of *Bythotrephes* increased. In spite of these changes, energy density (joules per gram) of the summer (June–August) diet has not changed since 1961. However, during the late-summer and fall, predation on *Bythotrephes*, and therefore the proportion of indigestible spines in the stomach, increases. Using bioenergetics models, we show that Lake Erie rainbow smelt could achieve 66%–155% greater growth between June and October if *Bythotrephes* spines were replaced with digestible prey items, resulting in end-of-season weights and lengths similar to the 1985–1990 period. Our results indicate that indigestible *Bythotrephes* spines may reduce growth by occupying space in the stomach but providing no nutritional value to the fish, thereby reducing the realized daily ration for rainbow smelt.

Résumé : Le régime alimentaire des éperlans arc-en-ciel (*Osmerus mordax*) de l'est du lac Érié s'est modifié depuis les années 1960, reflétant ainsi les changements des ressources alimentaires dus aux réductions du phosphore et à l'invasion d'espèces exotiques (les mollusques dreissénidés et *Bythotrephes longimanus*). Comme la croissance des éperlans arc-en ciel est plus faible dans les années 1990 que dans les années 1960, nous avons examiné le rôle possible des changements de régime alimentaire dans ce phénomène. Il s'est produit une diminution de la proportion du zooplancton dans le régime alimentaire d'été (mai à août) des poissons de deux classes de taille (longueurs à la fourche, 63–88 mm et 89–114 mm) entre 1961 et 1999. La proportion de *Bythotrephes* dans le zooplancton a augmenté. Malgré ces changements, la densité énergétique (joules par gramme) du régime d'été (juin à août) n'a pas changé depuis 1961. Cependant, durant la fin de l'été et l'automne, la consommation de *Bythotrephes* augmente et, par conséquent, il y a une plus grande proportion d'épines indigestes dans l'estomac. Des modèles bioénergétiques montrent que les éperlans arc-en-ciel du lac Érié pourraient atteindre une croissance de 66 à 155 % plus élevée entre juin et octobre si les épines de *Bythotrephes* étaient remplacées par des proies digestibles, ce qui donnerait des masses et des longueurs à la fin de la saison semblables à celles observées en 1985–1990. Nos données indiquent que les épines non assimilables de *Bythotrephes* peuvent réduire la croissance en occupant de l'espace dans l'estomac sans apporter de bénéfice nutritionnel au poisson et en réduisant ainsi la ration quotidienne réalisée de l'éperlan arc-en-ciel.

[Traduit par la Rédaction]

Introduction

Growth rates of eastern Lake Erie rainbow smelt (*Osmerus mordax*) began to decline in the 1960s (Henderson and Nepszy 1989). During the early 1980s, yearling size continued to decrease but also showed a strong 2-year cycle associated with yearling abundance (Einhouse et al. 1998). This pattern continued until 1994 when the 2-year cycle disappeared and a progressive decrease in yearling size began (Einhouse et al. 1998; Johnson et al. 2000) (Fig. 1). Changes in the yearling rainbow smelt diet have been suggested as an explanation for their decreased growth (Dermott et al. 1999). Interestingly, young-of-the-year growth rates did not change during the same time period (Fig. 1).

Three important ecosystem changes, with the potential to affect the rainbow smelt prey base, have occurred in eastern Lake Erie since rainbow smelt growth began to decline in the 1960s: reductions in phosphorus, the invasion of *Bythotrephes longimanus*, and the invasion of dreissenid mussels (*Dreissena polymorpha* and *Dreissena bugensis*).

Reductions in phosphorus concentrations began in 1972 and continued until the late 1980s (Dolan 1993), decreasing the productivity of the system. As a result, zooplankton production and mean body size decreased (Johannsson et al.

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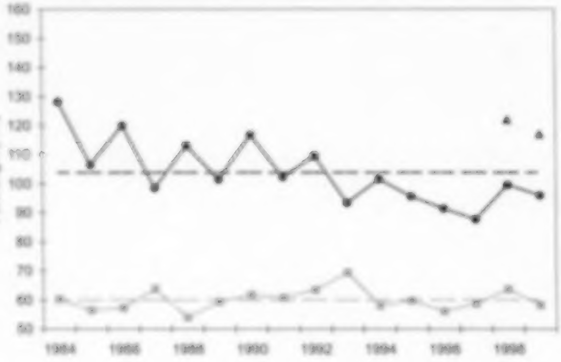
Fig. 1. Fall fork lengths of yearling (solid circles) and young-of-the-year (shaded squares) rainbow smelt from Ontario Ministry of Natural Resources 10-m index trawl in Long Point Bay, Ontario, 1984–1999. Figure modified from Johnson et al. (2000). Long-term average line for yearling (black broken line) and young-of-the-year (shaded broken line) rainbow smelt added. Results from predicted growth bioenergetic simulations (solid triangles) are presented for 1998 and 1999.



2000) and the community shifted toward more calanoid copepods relative to other crustaceans (Johannsson et al. 1999). A significant decrease in rainbow smelt abundance also occurred during this time period (Ryan et al. 1999).

In 1985, as phosphorus levels were near target levels (Dolan 1993), the exotic cladoceran *B. longimanus* was confirmed in the eastern basin zooplankton assemblage (Bur et al. 1986). In spite of its large indigestible caudal spine, it is a major prey item for rainbow smelt (Parker et al. 2001).

Dreissenid mussels were found in the eastern basin in 1989 (Dermott et al. 1993) and had little effect on the rainbow smelt prey base. Zooplankton biomass in the offshore region where rainbow smelt forage did not change significantly following the dreissenid invasion (Johannsson et al. 1999, 2000). In the profundal zone of eastern Lake Erie, density of nondreissenid benthic invertebrates increased, and biomass of nondreissenid benthic invertebrates decreased, following the dreissenid invasion (Dermott and Kerec 1997). Biomass and density of nondreissenid benthic invertebrates did not change further between 1993 and 1998 (MacDougall et al. 2001), although the spatial distribution of benthic invertebrates may have changed in the presence of dreissenid druses (Botts et al. 1996; Dermott and Kerec 1997).

Reduced rainbow smelt growth could result from changes in prey quantity or type. Lower daily consumption, decreased energy density of prey, or the expenditure of additional energy to find scarce prey items would reduce growth. Decreased growth could also result from the loss of large prey items that were previously important to rainbow smelt as they grew (Dermott et al. 1999). Although these are likely explanations for decreased growth during phosphorus reductions in the 1960–1980s, rainbow smelt growth continued to decline through the 1990s in spite of reduced smelt density (Ryan et al. 1999) and a relatively stable invertebrate prey base (Dermott et al. 1993; Johannsson et al. 1999; MacDougall et al. 2001).

The objective of this study was to examine alternative explanations for decreased rainbow smelt growth during the 1990s focusing on changes in composition and energy density of the diet. Our approach used rainbow smelt diet data from 1961, 1994, 1998, and 1999. Specifically, we (*i*) examined the summer (May–August) diet of two size classes to determine whether significant shifts in prey composition or energetic content have occurred as a result of ecosystem change and (*ii*) constructed a seasonal diet for 1998 and 1999 to determine if indigestible *Bythotrephes* spines found in rainbow smelt stomachs decreased the energy density of the diet enough to reduce seasonal growth.

Materials and methods

1998 and 1999 rainbow smelt collection and diet analyses

Rainbow smelt were obtained from 35 midwater trawl samples collected in 1998 (11 June, 2 July, 1 August, 6 October) and 1999 (6 June, 7 July, 3 October) in the eastern basin of Lake Erie. All fish were from night or sunrise collections with the exception of two daytime collections in June 1998. Nighttime collections were made in June between 2 h 10 min and 8 h 52 min (sunrise) after sunset, in July–August between 2 h 24 min and 8 h 20 min after sunset, and in October between 2 h 24 min and 10 h 52 min after sunset. Daytime collections were made in early afternoon. Trawling was concentrated in the southern half of the basin due to hazards such as gas wells and commercial fishing gear in Canadian waters. A four-seam midwater trawl with 6-mm2 cod-end mesh and average fishing dimensions of 6.0 m vertical gape and 6.0 m horizontal spread was used. Trawls lasted 20 min at an average velocity of 1.5 m·s^{-1}. A subsample of yearling and older fish was measured for fork length (millimetres) prior to processing specimens for diet analysis. From each trawl catch, we randomly selected 50 rainbow smelt from the yearling size class (≤120 mm) and actively selected up to 50 larger (>120 mm) rainbow smelt to ensure that all life stages were represented in the diet analyses. The body cavity of each fish was slit prior to preservation in 95% ethanol.

At the laboratory, rainbow smelt from each trawl were processed until at least 20 fish (10 yearling size class, 10 larger size class) with stomach contents, not including those containing only *Bythotrephes* spines, were examined. Fork length and total length measurements were made to the nearest millimetre on each fish. The stomach was then removed and assessed for degree of fullness based on a modification of the Hynes–Thompson method (Hynes 1950; Thompson 1959), a volumetric diet estimate that assigns points to prey items found in the stomach. Each stomach was emptied into a petri dish and inspected under a dissecting scope to ensure that no food items were missed. Prey items were identified to major groups, calanoid copepods, cyclopoid copepods, unknown copepods or copepodites, nauplii, *Bosmina* or *Eubosmina*, *Daphnia*, *Bythotrephes* bodies, *Bythotrephes* spines, benthic or emergent invertebrates, fish, and unknown or digested material, and assigned a proportion of the degree of stomach fullness based on their estimated volume. *Bythotrephes* spines and *Bythotrephes* bodies were considered separately because of the longer retention time of caudal spines in rainbow smelt (Parker et al. 2001). Because of classification differences among studies, prey categories were grouped as copepods, cladocerans, *Bythotrephes* bodies, *Bythotrephes* spines, benthos, fish, and unknown or digested material. The proportion of unknown or digested material (ranging from 0.01 to 0.11) was partitioned among all prey categories except *Bythotrephes* spines. Zooplankton proportion was defined as copepods plus cladocerans including *Bythotrephes*.

1961 and 1994 rainbow smelt collections and diet analyses

Rainbow smelt from the 1961 (Bidgood 1961) and 1994 (Dermott et al. 1999; L.D. Witzel, unpublished data) studies used fish from the central and eastern basins of Lake Erie. In 1961, central basin rainbow smelt were collected with a 12-mm cod-end midwater trawl and eastern basin smelt were collected with a commercial Texas bottom trawl. The 1994 collections (2 May, 2 July) were made in the eastern and central basins using commercial Texas bottom trawls or a standard 10-m index trawl. In both 1961 and 1994, collections were primarily made during early daylight hours. During both years, specimens were packed on ice immediately upon landing and transferred to freezers upon arrival at the dock. In 1961 and 1994, the Hynes–Thompson method (Hynes 1950; Thompson 1959) was used to estimate the volumetric composition of the diet.

Summer diet of rainbow smelt (1961–1999)

Summer diet information for individual yearling and older rainbow smelt was available between the last week of May and the first week of August for 1994, 1998, and 1999. Bidgood (1961) generated size-class diets based on an amalgamation of data for May, June, July, and August. Of the fish examined by Bidgood (1961), 67% (1306 of 1936) of all fish with stomach contents and 72% (19 100 of 26 678) of all prey points assigned under the Hynes–Thompson method (Hynes 1950; Thompson 1959) were obtained between the last week of May and the first week of August. Diet data were available in 2-week intervals for the 1961 and 3- to 4-week intervals for 1994, 1998, and 1999. Fork length categories were defined at natural breaks in the diet identified by Bidgood (1961) at 63–88 and 89–114 mm. To remain consistent with methodologies used in Bidgood (1961), averages were calculated based on diets for individual fish. A May–August summer diet was determined by calculating a grand mean proportion for each prey type from the mean proportion of each prey type from individual months in rainbow smelt diets from 1994, 1998, and 1999.

Generalized linear models, with a binomial family and logit link, and *F* tests were used to test the significance of year as (*i*) a predictor of the proportion of zooplankton in the summer diet of rainbow smelt in 1961, 1994, 1998, and 1999 and (*ii*) a predictor of the proportion of *Bythotrephes* in the summer diet of rainbow smelt in 1994, 1998, and 1999. Two size classes of rainbow smelt were included for each year in these analyses. As differences between size classes were expected, the effect of year was tested in the presence of size class. All analyses were completed with S-Plus (Math-Soft, Inc. 1999) and considered significant at the $p <$ 0.05 level. A mean percent (±2 SE) of summer stomach fullness (including empty stomachs) was calculated for both rainbow smelt size classes in 1994, 1998, and 1999 using individual trawls. Results were compared with a mean value calculated from Bidgood (1961) for all size classes during the same time period.

A comparison was made of the energy density of the summer diet of rainbow smelt in 1961, 1994, 1998, and 1999. Summer diets for the two rainbow smelt size classes were taken from the previous analysis. All prey energy density values were based on Cummins and Wuycheck (1971) as implemented in prior bioenergetics models. Prey energy densities for fish (4435 $J \cdot g^{-1}$), benthos (3138 $J \cdot g^{-1}$), and *Bythotrephes* (1674 $J \cdot g^{-1}$) were taken from Lantry and Stewart (1993). However, because Lantry and Stewart (1993) used an amalgamated energy density for copepods and cladocerans, individual energy densities for copepods (2300 $J \cdot g^{-1}$) and cladocerans (1674 $J \cdot g^{-1}$) were based on Stewart and Binkowski (1986). As *Bythotrephes* spines are indigestible, we assigned spines an energy density of 0 $J \cdot g^{-1}$. The energy density of the summer diet for both rainbow smelt size classes was calculated by multiplying the proportion of each diet item by its energy density.

A generalized linear model was used to test the significance of year as a predictor of summer diet energy in two size classes of rainbow smelt in 1961, 1994, 1998, and 1999. As differences between size classes were expected, the effect of year was tested in the presence of size class.

Temporal patterns in stomach fullness during 1998 and 1999 were considered by expressing mean percentage of stomach fullness (including empty stomach) for each trawl sample as a function of time of collection in minutes after sunset. Only those midwater trawls performed between dusk and dawn were included in the analysis. The relationship between stomach fullness and time of collection was investigated with a correlation analysis. Data were also plotted and fit with a second-order polynomial curve, as rainbow smelt are expected to have their highest feeding rates at dusk and dawn crepuscular periods.

Energetics of *Bythotrephes* spine consumption (1998 and 1999)

The potential effect of *Bythotrephes* spines on yearling rainbow smelt growth (1 June – 31 October) in 1998 and 1999 was investigated using a bioenergetics model. The mean fork lengths of yearling rainbow smelt were calculated from midwater trawl samples for June, July–August, and October of 1998 and 1999. For diet calculations, the size ranges were defined as the mean yearling fork length ± 5 mm. As no diet information was available for September, the results of the July–August diet were used for 1–15 September and the results of the October diet were used for 16–30 September. Kozuchowski (1996) found increased consumption of *Bythotrephes* by rainbow smelt during the month of September, a condition that is similar to October diet observations in our study.

Fish Bioenergetics 3.0 (Hanson et al. 1997) was used for the 1 June – 31 October growth simulations. Model parameters, including the summer simulation temperature value of 10 °C, were taken from Lantry and Stewart (1993). Weights for 1 June and 31 October of each year were calculated from the mean fork length in June and October of each year using a wet weight (grams) versus fork length (millimetres) regression equation developed for eastern Lake Erie rainbow smelt from 1984–2001 (L.D. Witzel, unpublished data). The regression was

$$\log_{10}(\text{wet weight}) = 3.16 \log_{10}(\text{fork length}) - 5.52 \quad (R^2 = 0.93, N = 4428)$$

Given the observed start and end weights and the observed diet, the year-specific simulations were run to determine the proportion of maximum consumption (p value) needed for rainbow smelt to achieve the observed growth in 1998 and 1999. Diet proportions for these simulations included the indigestible *Bythotrephes* spines with an energy density of 0 $J \cdot g^{-1}$. Seasonal diet proportions and the total energy density of the diet are presented in Table 1.

A second set of year-specific bioenergetic simulations was used to predict rainbow smelt growth if the indigestible *Bythotrephes* spines were replaced with nutritional prey items. *Bythotrephes* spines were reassigned an energy density value equivalent to the mean of other prey items weighted by their proportion in the diet in a given month. The models were initiated with the observed starting weight on 1 June and run to 31 October, using the p values determined from the first bioenergetics simulations, to predict the new seasonal weight gains. Predicted weight gains were then converted to their corresponding fork length using the length–weight regression equation presented above for comparison with observed growth.

Results

Summer diet of rainbow smelt (1961–1999)

The generalized linear models indicated that the proportion of zooplankton in the diet of rainbow smelt has decreased since 1961. Year was a significant predictor of zooplankton proportion in the diet ($p = 0.05$, $F = 9.56$, $N = 8$). In the second generalized linear logistic model, year was a significant predictor of *Bythotrephes* proportion in the diet ($p = 0.01$, $F = 70.06$, $N = 6$). In both analyses, size class was retained as an explanatory variable even though it was not significant ($p > 0.07$) to remove any effect of size class on the analyses. Mean percentage of summer stomach fullness (± 2 SE) for 63- to 88-mm fork length rainbow smelt in 1994 (46 ± 28, $N = 5$), 1998 (59 ± 14, $N = 13$), and 1999 (62 ± 10, $N = 12$) and for 89- to 114-mm fork length rainbow smelt in 1994 (40 ± 26, $N = 4$), 1998 (52 ± 14, $N = 13$), and 1999 (55 ± 12, $N = 8$) did not differ significantly from the mean percentage of fullness of 66 calculated for the same time period in Bidgood (1961). The total number of specimens examined by Bidgood (1961) was 1388; however, no sample sizes were given for individual time periods and the data were an amalgamation of all size classes.

The energy densities (joules per gram) of rainbow smelt diets in 1994, 1998, and 1999 were not significantly different from those of the 1961 diet. After controlling for the effect of size class, year was not a significant predictor of energy density in the diet ($p = 0.41$, $F = 1.31$, $N = 8$). Size class was retained as an explanatory variable even though it was not significant ($p > 0.21$) to remove any effect of size class on the analysis. The diets of the 63- to 88-mm fork length rainbow smelt in 1994, 1998, and 1999 were within 6% of the energy density of the 1961 diet. The diets of 89-

Table 1. Proportion of fullness by volume of diet items and mean energy density used in 1998 and 1999 Lake Erie yearling rainbow smelt bioenergetics simulations (1 June – 31 October).

Date	Size range (mm)	N	Copepods	Cladocerans	*Bythotrephes* bodies	*Bythotrephes* spines	Benthos	Fish	Energy ($J \cdot g$ wet weight^{-1})	Energy* ($J \cdot g$ wet weight^{-1})
1998										
1 June – 30 June	82–92	71	0.64	0.09	0.04	0.02	0.21	0.00	2349	2403
1 July – 15 Sept.	88–98	16	0.19	0.17	0.39	0.11	0.00	0.14	1995	2247
16 Sept. – 31 Oct.	99–109	20	0.09	0.01	0.47	0.43	0.00	0.00	1010	1774
1999										
1 June – 30 June	67–77	40	0.47	0.31	0.00	0.00	0.22	0.00	2299	2299
1 July – 15 Sept.	79–89	30	0.33	0.21	0.32	0.10	0.04	0.00	1772	1964
16 Sept. – 31 Oct.	94–104	31	0.18	0.02	0.30	0.29	0.14	0.07	1699	2399

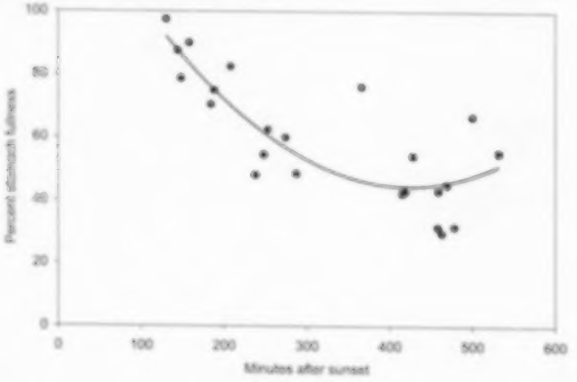
Note: Energy of the diets with 0 $J \cdot g$ wet weight^{-1} spines for observed growth (Energy) and for simulations with spines assigned the mean energy density of other items in the diet for predicted growth (Energy*) is presented. N is the number of individual fish.

Table 2. Proportion of fullness by volume of diet items and mean energy density for two size classes (63–88 and 89–114 mm fork length) of Lake Erie rainbow smelt in summer (May–August) of 1961, 1994, 1998, and 1999.

Year	Size range (mm)	N	Copepods	Cladocerans	*Bythotrephes* bodies	*Bythotrephes* spines	Benthos	Fish	Energy (J·g wet weight^{-1})
1961	63–88		0.78	0.19	0.00	0.00	0.03	0.00	2206
	89–114		0.14	0.75	0.00	0.00	0.08	0.03	1962
1994	63–88	77	0.79	0.13	0.00	0.00	0.08	0.00	2286
	89–114	110	0.57	0.13	0.01	0.00	0.29	0.00	2455
1998	63–88	82	0.42	0.26	0.19	0.03	0.06	0.04	2085
	89–114	76	0.35	0.19	0.21	0.06	0.14	0.05	2136
1999	63–88	135	0.43	0.24	0.15	0.04	0.14	0.00	2081
	89–114	54	0.03	0.10	0.19	0.11	0.55	0.02	2369

Note: Indigestible *Bythotrephes* spines were assumed to have no digestible energetic value (0 J·g wet weight^{-1}). N is the number of individual fish. The total number specimens examined by Bidgood (1961) was 1388; however, no sample sizes were given for individual size classes.

Fig. 2. Percent stomach fullness for rainbow smelt in 1998 and 1999 as a function of time of collection (minutes after sunset). A second-order polynomial line ($y = 0.0006x^2 - 0.4693x + 143.77$; $R^2 = 0.69$, $N = 23$ trawls) is fit to the data, as peak feeding is expected at sunset and sunrise.



to 114-mm rainbow smelt in 1994, 1998, and 1999 were within 25% of the energy density of the 1961 diet (Table 2).

Stomach fullness of rainbow smelt in 1998 and 1999 was significantly negatively correlated with time of collection ($p < 0.01$, $r = -0.76$, $N = 23$). Stomach fullness was highest near sunset, decreased through the night, and began increasing near dawn (Fig. 2). The data were fit with a second-order polynomial curve ($R^2 = 0.688$, $N = 23$) (Fig. 2).

Energetics of *Bythotrephes* spine consumption (1998 and 1999)

The bioenergetic simulations for observed growth provided consumption estimates for seasonal growth in 1998 and 1999 with 0 J·g^{-1} *Bythotrephes* spines in the diet. A p value of 0.58 was calculated for the 1998 simulation in which rainbow smelt fork length increased from 87 to 104 mm over the season, an increase in wet weight from 4.1 to 7.2 g. Similarly, a p value of 0.60 was calculated for the 1999 simulation in which rainbow smelt fork length increased from 72 to 99 mm, an increase in wet weight from 2.2 to 6.2 g.

We also used the energetics model to predict growth of rainbow smelt when *Bythotrephes* spines were assigned a weighted energy density based on other prey items in the diet. In the 1998 predicted growth simulation, rainbow smelt wet weight increased over the season from 4.1 to 12.0 g, an increase in fork length from 87 to 122 mm. These results were 155% (weight) and 106% (length) greater than observed growth when *Bythotrephes* spines had 0 J·g^{-1}. In the 1999 predicted growth simulation, rainbow smelt wet weight increased over the season from 2.2 to 10.5 g, an increase in fork length from 72 to 117 mm. The 1999 simulation results were 108% (weight) and 67% (length) greater than for observed growth in the 0 J·g^{-1} simulation. Total energy density in the diet during predicted growth simulations was greater than for those that used observed growth in all months except June 1999 when no *Bythotrephes* were present in the diet (Table 1).

Discussion

The composition of the diet of eastern Lake Erie rainbow smelt has changed since the 1960s; however, these changes have not decreased average energy density of the summer diet or the ability of rainbow smelt to fill their stomachs during peak feeding.

The proportion of zooplankton in the rainbow smelt summer diet has decreased and trends in the diet parallel changes that occurred in eastern Lake Erie associated with decreasing phosphorus levels (Dermott et al. 1999), the invasion of dreissenids, and the invasion of *Bythotrephes*. The 1960s diet of 89- to 114-mm rainbow smelt had a high proportion of cladocerans and reflected the dominance of the eastern basin ecosystem by large-bodied cladocerans and cyclopoid copepods (Davis 1968; Patalas 1972). Following phosphorus reductions, the eastern basin ecosystem shifted toward a higher number of calanoid copepods relative to other crustaceans (Johannsson et al. 1999) and copepods subsequently replaced cladocerans as the dominant prey item of rainbow smelt. A greater proportion of benthic or emergent invertebrates is present in the 1990 diets than in the 1961 diets of both size classes. Increased exploitation of both benthic and ephemeral prey items, such as chironomid pupae, may have resulted from increased water clarity (Mayer et al. 2001) or localized concentrations of benthic prey associated with dreissenid druses (Botts et al. 1996). In their review of the influence of zebra mussels on European aquatic systems, Karatayev et al. (1997) found no evidence of dreissenid effect, positive or negative, on planktivorous fish species. However, evidence indicates that fish capable of benthic feeding may be positively affected by an increased biomass of benthic invertebrates associated with zebra mussels (Karatayev et al. 1997).

The proportion of *Bythotrephes* in the diet of eastern Lake Erie rainbow smelt increased from 1994 to 1998–1999. Interestingly, density estimates for *Bythotrephes* are consistently low (e.g., Johannsson et al. 1999). Several explanations for the apparent increase in consumption of *Bythotrephes* by rainbow smelt are possible. The reduction in the size of other zooplankton prey items (Johannsson et al. 1999), or increase in water clarity that enhances visibility, may make *Bythotrephes* a more attainable prey item for rainbow smelt. Alternatively, *Bythotrephes* densities may be poorly represented by conventional zooplankton sampling methods and may have increased more than suggested by the available data. *Bythotrephes* move rapidly and may avoid sampling gear (Johannsson et al. 1999). Studies in European lakes suggest that *Bythotrephes* may be undetectable in zooplankton tows but present in the stomachs of fish (e.g., de Bernardi 1981; Manca and Ruggiu 1998).

The compositional shift in the summer (May–August) diet of rainbow smelt during the 1990s did not significantly change energy density of the diet as compared with 1961. In spite of the presence of *Bythotrephes*' indigestible caudal spine, the summer diet of the 89- to 114-mm rainbow smelt size class during the 1990s contained between 8% and 25% more energy per unit volume than the diet in 1961. This is due to the inclusion of more energy-rich benthic or emergent invertebrates and fish in the 1990s summer diets of both size classes. Thus, decreased growth and condition of yearling rainbow smelt in eastern Lake Erie through the 1990s does not appear to be caused by decreased energy density of the summer diet.

Although the summer diet analysis suggested that indigestible *Bythotrephes* spines were not compromising the energy density of the summer diet in the 1990s, consumption of *Bythotrephes* increases dramatically late in the growing season (Parker et al. 2001). It is also during the late summer and early fall that *Bythotrephes* spines are differentially retained, being present in higher numbers than would be expected based on the number of *Bythotrephes* bodies (Parker et al. 2001). Indigestible spines accounted for 43% and 29% of yearling rainbow smelt stomach volume in October of 1998 and 1999, respectively. If rainbow smelt were able to replace *Bythotrephes* spines with prey items of the average energy density observed in the diet, the same rate of consumption (p value) would predict growth for yearling rainbow smelt to be 2–2.5 times greater than observed growth. Our p values (~0.60) suggest relatively high food intake, as wild fish seldom consume prey at rates higher than 30%–40% of maximum rates observed in the laboratory (Walters 2000). Similarly, our p values are equivalent to those found for fast-growing largemouth bass (*Micropterus salmoides*) (Irwin et al. 2003) and higher than those found for bloater (*Coregonus hoyi*) (Rudstam et al. 1994). Predicted seasonal weight gains of 7.9 g (1998) and 8.3 g (1999) with final fall weights of 12.0 g (1998) and 10.5 g (1999) were similar to observed growth in the 1985–1990 period (Dermott et al. 1999). Predicted fall lengths of 122 and 117 mm are similar to those observed for yearling rainbow smelt during 1984–1990 (Dermott et al. 1999).

Bythotrephes spines will only affect rainbow smelt growth if their presence in the gut decreases intake of nutritional food. If food availability has declined, rainbow smelt may not be able to find additional food. Results indicate that, although variable, the mean percentage of stomach fullness for rainbow smelt in both size classes in 1994, 1998, and 1999 was not significantly different than the mean value for the same time period in 1961. Variability in data from 1994, 1998, and 1999 likely resulted from sampling across periods of low and peak feeding. Rainbow smelt caught shortly after dusk had percent stomach fullness values of 80%–100%, suggesting that food was not limiting peak feeding. Such discrete diel peaks in feeding rates are common in many fish species including smelt (Ferguson 1965; Walters 2000). Thus, spines present in the stomach will likely decrease the quantity of digestible food present in the stomach during peak dusk and dawn feeding periods. Our analysis shows that if these fish could replace the *Bythotrephes* spines with nutritional prey, their growth rate would be similar to observed growth prior to the *Bythotrephes* invasion. This is, of course, only one of several hypotheses for effects of diet on growth rates. It is also possible that *Bythotrephes* are harder to catch and therefore require increased activity by the fish, that the energy density of benthic and (or) zooplankton prey has decreased over time, or that the nutritional quality rather than energy has decreased. However, if the energetic or nutritional quality of benthic or zooplankton prey has decreased, the growth rates of young-of-the-year rainbow smelt should also have decreased. This has not been observed. Fall fork length of young-of-the-year rainbow smelt has remained relatively constant since 1984. Young-of-the-year rainbow smelt also prey upon *Bythotrephes* during the fall but consume far fewer individuals than yearling and older rainbow smelt and contain significantly fewer excess *Bythotrephes* spines (Parker et al. 2001).

Giussani and de Bernardi (1977) observed a similar trend in *Bythotrephes* consumption and coregonid growth in Lago Maggiore. Although *Bythotrephes* had the highest energy density per individual of all zooplankton prey species in Lago Maggiore, the assimilation efficiency of this prey item was significantly lower than that of other prey types. *Coregonus* species were unable to use many of the ingested *Bythotrephes* calories, and it was during the midsummer to early-fall period of high predation on *Bythotrephes* that *Coregonus* species had the lowest seasonal growth rate (Giussani and de Bernardi 1977). However, in Harp Lake, Ontario, a system invaded by *Bythotrephes*, Coulas et al. (1998) found no decrease in lake herring (*Coregonus artedi*) condition as compared with neighboring uninvaded lakes. The comparison of growth rates between invaded and uninvaded lakes may have been complicated by differences in the abundance of other preferred prey types in the zooplankton assemblages of individual lakes (Coulas et al. 1998). Similarly, seasonal patterns in growth may not have been apparent, as collections for herring condition took place between mid-April and late May (Coulas et al. 1998), prior to the period of highest predation on *Bythotrephes*.

Collection methods differed between past and present

Lake Erie rainbow smelt diet studies. In particular, rainbow smelt were collected during the day with bottom trawls in 1961 (Bidgood 1961) and 1994 (Dermott et al. 1999) and during the crepuscular period and at night with midwater trawls in 1998 and 1999. However, we felt that the comparison of diet information was acceptable for several reasons. Differences in diel composition of rainbow smelt diets are particularly large in systems with abundant *Mysis relicta* (e.g., Urban and Brandt 1993); however, eastern Lake Erie has few *Mysis* (Johannsson et al. 1999). Additionally, the proportion of zooplankton in the diet of eastern basin rainbow smelt will increase at night owing to increased overlap with zooplankton prey items resulting from the crepuscular vertical migration of yearling and older rainbow smelt. Our conclusion that energy density in the summer diet of rainbow smelt has not declined will therefore be robust, as nighttime midwater collections of rainbow smelt will provide liberal estimates of zooplankton consumption (with lower energy density) and conservative estimates of benthos consumption (with higher energy density) relative to daytime samples.

Changes in the composition of the summer (May–August) diet of rainbow smelt since the 1960s do not explain the continued decline in rainbow smelt growth through the 1990s. Our analyses indicated that the energy density of the rainbow smelt summer diet during the 1990s was not significantly different from that in 1961 and that prey availability was not limited. Our bioenergetics results indicate that indigestible *Bythotrephes* spines may reduce seasonal growth by occupying space in the stomach but providing no nutritional value to the fish, thereby reducing the realized daily ration for rainbow smelt. Other explanations are possible and should be explored.

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Impacts of altered benthic invertebrate communities on the feeding ecology of yellow perch (*Perca flavescens*) in metal-contaminated lakes

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Abstract: Metal contamination can disrupt trophic links in food webs by altering the taxonomic composition and size structure of benthic macroinvertebrate communities. Benthic macroinvertebrates and yellow perch (*Perca flavescens*) were collected from six lakes along a gradient of cadmium (Cd) and copper (Cu) contamination in Quebec, Canada. The two most contaminated lakes had significantly lower densities of several benthic macroinvertebrate taxa and significantly lower Shannon's index than less contaminated lakes. The stomach contents of perch from the most contaminated lakes were less diverse, with a greater reliance on chironomids and (or) zooplankton than perch from other study lakes. The size of prey in perch from the most contaminated lakes did not increase with age and the mean prey size was smaller than in other, less contaminated lakes. Perch from lakes with medium to low levels of contamination weighed significantly more than perch from lakes with high levels of contamination. This reduction in growth is attributed to the increased costs of foraging on a simplified prey base in metal-contaminated systems.

Résumé : La contamination par les métaux peut perturber les liens trophiques dans les réseaux alimentaires en modifiant la composition taxinomique et la structure en taille des communautés de macroinvertébrés benthiques. Nous avons prélevé des macroinvertébrés benthiques et des perchaudes (*Perca flavescens*) dans six lacs le long d'un gradient de contamination au cadmium (Cd) et au cuivre (Cu) au Québec, Canada. Dans les deux lacs les plus contaminés, la densité de plusieurs taxons de macroinvertébrés benthiques est significativement plus basse et l'indice de Shannon est significativement plus faible que dans les lacs moins contaminés. Les contenus stomacaux de perchaudes provenant des lacs les plus contaminés sont moins diversifiés et sont plus dominés par les chironomidés et (ou) le zooplancton que ceux des perchaudes des autres lacs étudiés. Dans les lacs les plus contaminés, la taille des proies n'augmente pas en fonction de l'âge des perchaudes et la taille moyenne des proies est plus faible que dans les autres lacs moins contaminés. Les perchaudes provenant des lacs à contamination moyenne à faible pèsent significativement plus que les perchaudes des lacs à forte contamination. Nous attribuons cette réduction de la croissance aux coûts accrus de la recherche de nourriture dans un réseau de proies simplifié dans les systèmes contaminés par les métaux.

[Traduit par la Rédaction]

Introduction

Many aquatic ecosystems in North America are chronically exposed to heavy metal contamination (Luoma 1989). Generally, ecotoxicologists assess the impact of these contaminants by one of two approaches: using standard toxicity tests (e.g., LC_{50}s) or by relating metal concentrations (or some physiological endpoint) in indigenous species to metal concentrations in the ambient environment. Field studies that have examined the effects of metal contamination on fish have generally found that as exposure increases among systems, there is an increase in physiological impairment (Laflamme et al. 2000) and accumulation of metals in organs like the liver (Harrison and Klaverkamp 1990; Amundsen et al. 1997). Relatively few studies have examined the effects of reduced taxonomic diversity in contaminant-exposed benthic invertebrate communities on food webs (Munkittrick and Dixon 1988; Clements and Rees 1997).

Many fish take advantage of resources in pelagic and benthic environments over the course of ontogeny. As fish grow, their dominant prey type changes (Mittelbach and Persson 1998). For example, young-of-the-year yellow perch (*Perca flavescens*) are predominantly pelagic foragers. By the end of their first summer, perch move into the littoral zone and feed on benthic invertebrates (Scott and Crossman 1973).

Ontogenetic changes between pelagic and benthic feeding are typically associated with increased prey size. Keast (1977) found that the size of prey in yellow perch increased progressively from 0.1 mg in young-of-the-year to 100–250 mg in

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Table 1. Limnological characteristics and metal concentrations in various compartments of the Rouyn-Noranda study lakes.

	Limnological parameters*			Aqueous metals*		Sediment metals†		Perch liver metals†	
Lake	[DOC] (mg·L^{-1})	[Ca^{2+}] (μmol^{-1})	[H$^+$] (nmol^{-1})	[Cd^{2+}] (nmol^{-1})	[Cu^{2+}] (nmol^{-1})	[Cd] (nmol·g^{-1})	[Cu] (nmol·g^{-1})	[Cd] (μg·g^{-1})	[Cu] (μg·g^{-1})
L1	7.7	340	6.0	0.01	<0.001	5.1	144.0	2.9	10.4
L2	9.5	290	32.0	0.02	<0.001	—	—	5.3	10.8
L3	9.0	130	110.0	0.16	0.006	10.0	143.0	20.3	20.4
L4	14.7	150	250.0	0.21	0.012	12.0	238.0	25.1	12.9
L5	3.5	950	630.0	1.40	0.630	590.0	105 000.0	45.7	246.5
L6	5.5	590	32.0	3.70	0.140	200.0	19 400.0	61.3	148.5

Note: Aqueous metals represent the free metal ion concentration. The free metal ion concentration was measured in the water column using dialysers installed at 10 cm above the sediments; see Fortin and Campbell (1998) and Tessier et al. (1993) for details of the methodology and quality control for aqueous and sediment metal analyses. Sediment concentrations were not measured in L2. DOC is the dissolved organic C concentration of the water column.

*Data from Campbell et al. (2003).

†Data from Laflamme et al. (2000).

the oldest age class. Similarly, perch growth rates are negatively correlated with the percent contribution of small prey in their diets (Boisclair and Rasmussen 1996). To maximize body size, fish need to maintain relatively high growth efficiency throughout ontogenetic niche shifts. Prey that are too small or too large relative to their predator's size will result in fish with low growth efficiencies. Benthic invertebrates are thus an important dietary stepping-stone between zooplanktivory and piscivory (Sherwood et al. 2002), as these organisms span a wide range of sizes (e.g., <0.5– 512 mg) (Hanson et al. 1989; Rasmussen 1993). This diversity of prey sizes allows predators to switch to more energetically beneficial prey before their growth efficiencies decrease.

Any perturbation to the food web that results in the loss of all or part of the benthic invertebrate community has the potential to severely alter the efficiency of energy acquisition of predators. For fish that have a benthivorous stage during ontogeny, the absence of a continuous range of benthic prey sizes can result in stunted fish (Heath and Roff 1996; Sherwood et al. 2002). From the perspective of an omnivorous fish, it is not necessarily the taxonomic identity of prey that is crucial for attaining maximum body size, but the availability of appropriate prey sizes. By changing the taxonomic diversity (Gower et al. 1994; Day et al. 1995; Clements et al. 2000), abundance (Clements et al. 1989), and size availability (Gaston et al. 1998) of the benthic invertebrate community, metal contamination has the potential to weaken benthic–pelagic coupling, forcing fish to rely more on less energetically efficient prey items like small benthic invertebrates.

Growth rates of organisms like fish are one of the most common indices of bioenergetic health in studies of pollution (Langston and Spence 1995). Although fish toxicologists often attribute poor growth to direct effects of toxic chemicals (i.e., physiological impairment prevents fish from maximizing energy allotted for growth; Munkittrick and McCarty 1995), fish growth in uncontaminated systems can be affected by alterations in the abundance, diversity, and size of benthic prey (Mittelbach 1981; Heath and Roff 1996; Cobb and Watzin 1998).

In this study, we sought to explore some of the effects of metal exposure on food webs along a gradient of contamination. We demonstrate that reduced taxonomic diversity of chronically metal-exposed benthic macroinvertebrate communities is linked to alterations in the diet patterns of a common fresh water fish, the yellow perch. Furthermore, we discuss how these changes have led to impaired growth in perch.

Methods

Study area

Rouyn-Noranda in northwestern Quebec, Canada, has a long history of mining activity that has led to the accumulation of metals in the water column and sediments of lakes downwind from the city's Cu refinery (Wang et al. 1999). A clear metal gradient for Cd, Cu, and Zn among lakes in this region has previously been established (Couillard et al. 1993; Wang et al. 1999; Perceval et al. 2002). In this study, we chose six lakes (48°00′N, 79°00′W) from this gradient. The six lakes differ substantially in their dissolved and sediment concentrations of metals but have little variation in their limnological characteristics (Table 1). Details of methodology and analysis of metals in sediment and water of these lakes can be found in Tessier et al. (1993) and Fortin and Campbell (1998). Two lakes, Opasatica (L1) and Dasserat (L2), are located approximately 35–40 km upwind from the refinery. Both are headwater lakes that have no anthropogenic source of contamination. The fish communities in these two lakes are relatively diverse and include suckers (*Catostomus* spp.), walleye (*Stizostedion vitreum*), northern pike (*Esox lucius*), yellow perch, pumpkinseed (*Lepomis gibbosus*), and a variety of littoral minnow species. Two lakes, Vaudray (L3) and Bousquet (L4), are located approximately 35–40 km downwind from the refinery and the only anthropogenic source of metal contamination is atmospheric deposition. These lakes have fish communities that are similar to those in L1 and L2. Lakes Osisko (south basin) (L5) and Dufault (L6) have the highest levels of contamination among the study lakes (Table 1). L5 was the mine-tailing pond for the refinery, although it no longer receives tailings and it is adjacent to the refinery in the city of Rouyn-Noranda. L6 has received mine drainage and atmospheric deposition of metals from the refinery and is located approximately 7 km north of the refinery. The fish community in L5 is entirely perch, while in L6, there are pike, suckers, and small forage fish (e.g., *Notropis* spp.). Lakes have been coded L1–L6 to reflect the general level of contamination, with L1 and L2 indicating the lowest levels, L3 and L4 indicating medium levels, and L5 and L6 indicating the highest levels of contamination.

Sampling and analysis

Yellow perch were collected from each of the six lakes in June of 2000. Each lake was sampled every day for 3 days for approximately 6 h (midafternoon until after dusk) each day. Young-of-the-year were collected using a mysis net (diameter 1 m, length 3.5 m, mesh size 250 µm) that was towed within and just outside the littoral zone after nightfall. The net was towed vertically and horizontally in a sine-like wave from the top of the metalimnion to just below the surface of the water. Other fish were collected using multimesh experimental gill nets (panel depth 1.5 m, mesh size ranging from 5 to 1.5 cm in 5-cm increments) and hook and line. Gill nets were set in parallel and perpendicular to the shoreline outside macrophyte beds for approximately 1 h per set. Perch were put on ice in the field and immediately frozen upon return to the laboratory. Wet weight and total length of each fish were measured in the laboratory. Opercula were used to identify the age of perch (Le Cren 1947).

Stable isotope analysis of dorsal white muscle tissue was used to establish a temporally integrated measure of diet patterns. Stable ^{13}C signatures of consumers generally reflect the same ^{13}C signature as their prey. As a result, ^{13}C signatures are conserved from the bottom of the food web to the top (Deniro and Epstein 1978; Vander Zanden and Rasmussen 1999) and can provide information about the original source of C within a food web. Consumers become enriched in ^{15}N relative to their prey by 3‰–4‰ (mean 3.4‰; Deniro and Epstein 1981). Stable ^{15}N signatures indicate trophic position within a food web (Vander Zanden et al. 1999). Zooplankton and benthic invertebrate samples were collected in June 2000 to determine their isotopic signatures. Zooplankton were collected from horizontal and vertical tows in the epilimnion with zooplankton nets (mesh size 250 µm). Benthic invertebrates were collected using a kicknet (45 × 23.5 cm) and an Eckman sampler (15.24 × 15.24 cm) for stable isotope analysis.

Whole invertebrates, pooled zooplankton, and perch muscle tissue were frozen, dried at 70 °C for 48 h in a drying oven, and ground into a fine powder using a mortar and pestle. Isotopic content was measured using a mass spectrometer at the G.G. Hatch Laboratories, University of Ottawa (Ottawa, Ontario). Trophic position was calculated as

$$(1)\qquad \text{Trophic position} = [(\delta^{15}N_{\text{consumer}} - \delta^{15}N_{\text{baseline}})/3.4‰] + 2$$

where $\delta^{15}N_{\text{baseline}}$ is the ^{15}N signature of the average benthic invertebrate (trophic position = 2) for each lake. Baseline signatures are used to express the trophic position of a consumer relative to the $\delta^{15}N$ of a primary consumer (Vander Zanden et al. 1999). We used the mean benthic invertebrate $\delta^{15}N$ as the baseline indicator. The value 3.4‰ represents the average trophic fractionation per trophic level. A trophic position of 4 or above is considered indicative of piscivory.

We used stomach contents and the mean weight of prey in the gut contents to further characterize the diet of perch. To measure the taxonomic richness of the perch population's diet, we measured the percent occurrence of prey items (percentage of fish/age category/lake with a particular prey item in their stomach contents; Hyslop 1980; Marshall and Elliott 1997). For each fish, we measured the total number of individuals of each prey item in the gut contents and the average dry weight of prey/fish (total weight of gut contents divided by the number of diet items) in the stomach contents. Stomach contents were dried in a drying oven at 70 °C for 48 h.

Benthic macroinvertebrates were collected from study lakes in June 1999 using a kicknet (45 × 23.5 cm) and an Eckman sampler (15.24 × 15.24 cm). Approximately 5 h were spent at each lake from early afternoon until early evening. We collected 15–24 samples from the littoral and sublittoral zones of each of the lakes. Samples were collected within macrophyte beds dominated by different species ("grassy" versus "broadleaf" macrophytes) and outside macrophyte beds to encompass as wide a range of habitats as possible. Kicknet samples were taken at depths <2 m and Eckman dredges were used for samples ≥2 m. Areas were converted to dimensions of square metres to estimate the number of invertebrates per square metre. Samples were sealed in bags, stored on ice, and frozen within 5 h of collection. We made no special effort to sample crayfish and unionid bivalves in this study, although the presence of unionid bivalves has been documented for L1–L4 (Wang et al. 1999). Benthic invertebrates were identified according to Peckarsky et al. (1990).

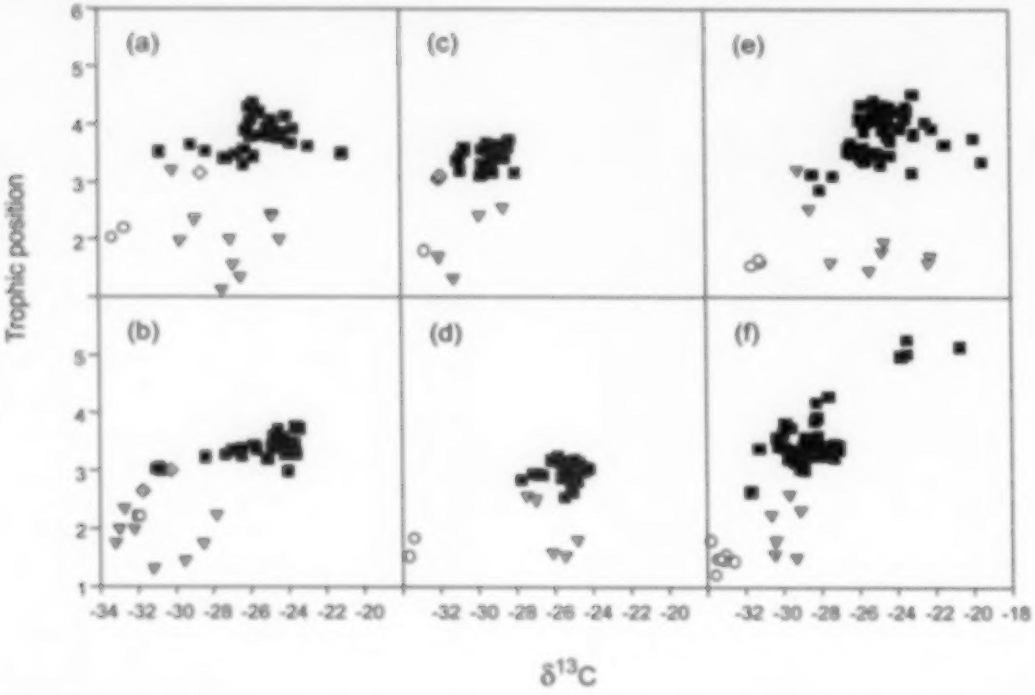
Statistical analysis

The relationship between fish growth and age was tested within lakes by ANCOVA, with fish weight as the dependent variable, age as the covariate, and sex as the factor (two levels, male and female). All weights were log-transformed to reduce the heteroscedasticity of the data. Differences in the maximum size of perch among lakes were tested by comparing the mean weights of the largest 25% of fish (fish weights in the 75th percentile). Mean weights were compared by one-way ANOVA followed by Tukey–Kramer's multiple comparison test to test for differences among lakes.

We used *t* tests to test for differences within lakes in the mean weight of piscivores (fish with trophic positions ≥4) and nonpiscivores (trophic position <4). Differences among lakes in the mean weight of piscivores were tested using one-way ANOVA followed by Tukey–Kramer's multiple comparison test.

Linear regression was used to test the relationship between the mean size of nonzooplankton prey in the stomach contents and age of perch within lakes. All weights were log-transformed to reduce heteroscedasticity in the data. The general linear model (GLM) procedure was used to test the relationship of mean weight of prey in the stomach contents and the weight of perch. The model incorporated an interaction term, which reflects the diversity of the in situ benthic invertebrate community (H-group with two levels, 1 for high diversity including L1–L4 and 2 for low diversity for L5 and L6). Shannon's index of diversity (*H'*) (Zar 1996) was used to compare average benthic community diversity among lakes. Differences in mean benthic invertebrate diversity were determined by one-way ANOVA followed by Tukey–Kramer's multiple comparison test. The group associations

Fig. 1. Trophic position relative to ^{13}C signature of yellow perch (*Perca flavescens*) (squares) from the Rouyn-Noranda study lakes (*a*) L1, (*b*) L2, (*c*) L4, (*d*) L3, (*e*) L5, and (*f*) L6 and their prey items: zooplankton (circles), benthic invertebrates (triangles), minnows (Cyprinidae; open diamond), and chaoborus (*Chaoborus* spp.; shaded diamonds).



of the Tukey–Kramer test were used to create the category H-group for the GLM.

All statistical analyses were done using SYSTAT 10.2. The level of significance was set at $p = 0.05$ for statistical tests. However, for the ANOVA testing differences in mean benthic invertebrate diversity, a more conservative p value was chosen using the Bonferroni correction ($p = \alpha/n = 0.008$).

Results

Yellow perch diet: stable isotopes

In L1, approximately 25% of the perch had a trophic position of ≥4, indicating a switch to piscivory in the adult population (Fig. 1). The trophic position of L2 perch never exceeded 4 (Fig. 1). L2 perch remain benthivorous (less negative δ^{13}C and trophic positions between 3 and 4 for all age classes past age 2+). The lack of age-1+ perch in L2 made it difficult to make inferences about their juvenile diet patterns.

The trophic position of perch from L3 and L4 never exceeded 4 (Fig. 1). The age-1+ and age-2+ perch in L3 and L4 had no consistent changes in trophic position with δ^{13}C signatures. There was considerable variability in the trophic position of age-1+ and age-2+ perch of these two lakes. As such, the isotopic signatures of age-1+ and age-2+ perch were not indicative of planktivorous feeding (low trophic position, lighter δ^{13}C) that progressed to benthic feeding.

In L5, approximately 25% of the perch had trophic positions ≥4 (Fig. 1), which typically indicates piscivorous feeding. All of the perch with trophic positions ≥4 were age 4+ or older. In L6, the majority of perch have the same trophic positions (between 3 and 4) (Fig. 1). A small number of perch in L6 have trophic positions ≥4 (Fig. 1). The trophic positions and δ^{13}C suggest that L6 fish were feeding on forage fish (e.g., spottail shiner (*Notropis hudsonius*)) that feed on epiphytic algae. The δ^{13}C range of L6 perch (from –21 to –31) indicates benthic feeding.

Yellow perch diet: stomach contents analysis

In lakes L1–L4, age-1+ diets showed a mixture of zooplankton feeding and benthic feeding (Table 2). In lakes L5 and L6, perch also had a mixture of zooplankton and benthic feeding, but the proportion of zooplanktivory in these two populations was higher than in L1–L4 (Table 2).

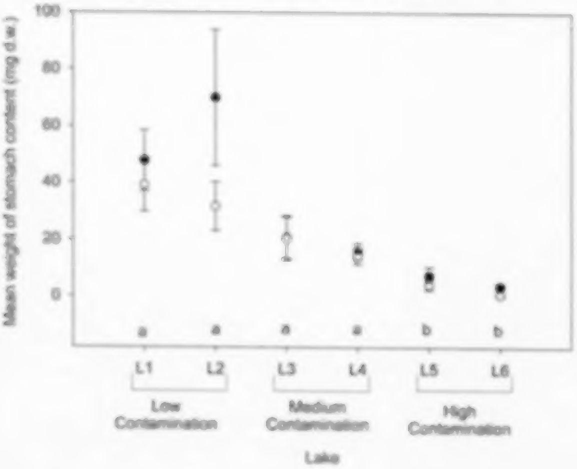
Benthic invertebrates dominated the stomach contents of age-2+ to age-3+ perch from L1–L4, particularly dipterans, trichopterans, and amphipods (Table 2). The benthivorous stage of age-2+ to age-3+ perch from L1 and L2 had relatively high taxonomic richness, and stomach contents contained large-bodied benthic taxa (e.g., odonates and crayfish). For example, 23% of age-2+ to age-3+ perch in L2 and 12% of ≥age-4+ perch consumed crayfish. The dominant prey for age-2+ to age-3+ perch in L3 and L4 were ephemeropterans. L5 perch did switch to benthivory at age 2+, but the benthic portion of their diet consisted only of dipterans (mostly Chironomidae larvae and pupae) and to a lesser degree trichopterans (mostly Leptoceridae). Unlike the perch from all of the other study lakes, almost all of the age-

Table 2. Percent occurrence (number of yellow perch (*Perca flavescens*) with that prey item in their stomach contents per age class) of prey for each perch age class in Rouyn-Noranda lakes.

Lake	Age (years)	*n*	ZOOP	DIP	EPH	ACA	ODO	TRI	AMP	Fish
L1	1+	8	75.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0
	2+–3+	4	0.0	75.0	25.0	25.0	50.0	25.0	25.0	0.0
	≥ 4 +	20	0.0	5.0	35.0	0.0	45.0	10.0	5.0	50.0
L2	1+	2	0.0	50.0	50.0	0.0	50.0	0.0	100.0	0.0
	2+–3+	17	11.8	29.4	17.6	0.0	58.8	41.2	58.8	5.9
	≥ 4 +	17	0.0	11.8	0.0	0.0	52.9	29.4	41.2	5.9
L3	1+	17	47.1	35.3	52.9	0.0	5.9	5.9	5.9	0.0
	2+–3+	11	0.0	9.1	72.7	0.0	18.2	0.0	18.2	0.0
	≥ 4 +	3	0.0	33.3	66.7	0.0	0.0	0.0	33.3	0.0
L4	1+	17	64.7	64.7	76.5	5.9	0.0	11.8	64.7	0.0
	2+–3+	8	25.0	37.5	75.0	0.0	0.0	0.0	50.0	12.5
	≥ 4 +	15	0.0	13.3	60.0	0.0	20.0	46.7	6.7	0.0
L5	1+	39	92.3	17.9	0.0	0.0	0.0	0.0	0.0	0.0
	2+–3+	11	0.0	72.7	0.0	9.1	9.1	36.4	0.0	0.0
	≥ 4 +	36	2.8	94.4	0.0	19.4	0.0	22.2	0.0	0.0
L6	1+	5	100.0	40.0	0.0	0.0	0.0	0.0	0.0	0.0
	2+–3+	35	94.3	37.1	0.0	0.0	0.0	2.9	0.0	2.9
	≥ 4 +	31	45.2	90.3	0.0	6.5	0.0	22.6	0.0	3.2

Note: ZOOP, zooplankton; DIP, Diptera larvae and pupae; EPH, Ephemeroptera; ACA, Acariformes (all Hydrachnidia); ODO, Odonata; TRI, Trichoptera; AMP, Amphipoda. Prey that only occurred in one or two lakes (Neuroptera % occurrence: L4, age 1+, age 2+ to age 3+, and ≥age 4+ = 5%, 12%, and 6%, respectively; crayfish: L4, age 4+ = 20%; L2, age 2+ to age 3+, and ≥age 4+ = 24% and 12%, respectively) or where items were always <5% occurrence (Coleoptera) were not included in the table.

Fig. 2. Mean (± SE) weight (mg dry weight (d.w.)) of stomach contents of yellow perch (*Perca flavescens*) in Rouyn-Noranda lakes. Solid circles are the average prey weights for the nonzooplankton prey. *n* = 25, 30, 16, 27, 42, and 38 for L1–L6, respectively. Open circles are the average prey weights for all of the prey in the stomach contents. L1, *n* = 34; L2, *n* = 31; L3, *n* = 32; L4, *n* = 39; L5, *n* = 79; L6, *n* = 70. Lakes with the same letter are not significantly different from each other (one-way ANOVA followed by a Tukey–Kramer multiple comparison test, $p = 0.05$). Tukey–Kramer group associations were the same whether or not zooplankton were included in the mean.



2+ to age-3+ perch (94.3%) in L6 had zooplankton in their stomachs. Only 37% of perch in L6 had dipterans and 3% trichopterans in their stomachs.

In L1 and L2, odonates were the dominant prey for perch ≥age 4+. L1 was the only lake where the perch demonstrated sustained piscivory (increasing in dominance through more than one age class). When the ≥age-4+ class was broken down by age (age 4+ to age 7+), fish were the third most important prey item for age-4+ perch in L1 and the most important for subsequent ages. Ephemeroptera were the dominant prey for perch in L3 and L4. By age 4+, L4 perch were feeding on crayfish (20%) in addition to Ephemeroptera and other large-bodied invertebrates like odonates. In L5, the diets of perch remain dominated by dipterans and to a lesser degree trichopterans. Although the proportion of zooplanktivory is lower in age-4+ perch from L5 and L6 than younger age classes in these lakes, it is a strikingly different pattern from that of the other lakes where zooplanktivory is entirely absent in older fish. In L6, almost half of age-4+ perch are still consuming zooplankton.

Perch from lakes with higher levels of contamination (L5 and L6) had significantly smaller prey in their stomachs than perch from lakes with lower levels of contamination. This pattern was the same whether or not zooplankton were included the analysis (mean prey size for all stomach contents: one-way ANOVA and Tukey–Kramer multiple comparison test, $F_{[5,279]} = 32.85$, $p < 0.\ 0001$; mean prey size for nonzooplankton stomach contents: one-way ANOVA and Tukey–Kramer multiple comparison test, $F_{[5,173]} = 5.94$, $p < 0.\ 0001$) (Fig. 2) As perch aged in L1–L4, the mean size of nonzooplankton prey increased (Table 3). Although the coefficients of determination (r^2) were not high (0.18–0.49), the

Table 3. Ontogenetic changes in the mean size of nonzooplankton prey in yellow perch (*Perca flavescens*) from Rouyn-Noranda lakes.

Lake	Regression equation	r^2	p	n
L1	log(prey weight) = –0.07 + 0.34age	0.27	0.005	28
L2	log(prey weight) = 0.14 + 0.30age	0.32	0.001	32
L3	log(prey weight) = 0.58 + 0.22age	0.18	0.021	30
L4	log(prey weight) = –0.15 + 0.22age	0.49	<0.001	39
L5	log(prey weight) = 0.04 + 0.05age	0.02	0.401	44
L6	log(prey weight) = –0.07 + 0.09age	0.04	0.236	40

Table 4. Relationship of log(fish weight) with age controlling for the effect of sex (two levels, male and female) for yellow perch (*Perca flavescens*) in Rouyn-Noranda lakes (ANCOVA).

			Factor (sex)		Covariate (age)	
Lake	r^2	n	F	p	F	p
L1	0.52	39	0.14	0.71	37.8	<0.001
L2	0.69	36	1.28	0.27	58.6	<0.001
L3	0.75	28	1.27	0.27	66.69	<0.001
L4	0.86	34	0.59	0.45	175.82	<0.001
L5	0.77	72	14.9	<0.001	189.70	<0.001
L6	0.60	77	1.27	0.27	66.69	<0.001

regressions were all significant for perch in L1–L4. Perch in L5 and L6 did not show any significant increase in mean prey size with age (Table 3).

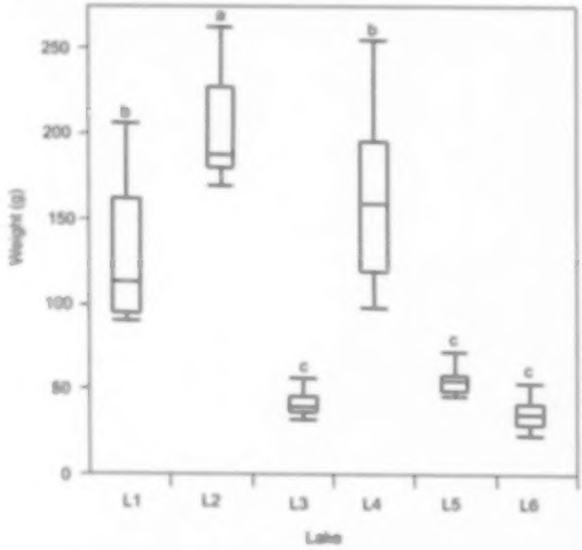
Yellow perch size

ANCOVA revealed that in all study lakes, perch weight increased significantly with age, and the influence of sex was nonsignificant in all lakes except L5 (Table 4). Perch in L2 were significantly larger than perch from other study lakes (one-way ANOVA followed by Tukey–Kramer multiple comparison test, $F_{[5,79]} = 83.16$, $p < 0.001$.) (Fig. 3) There were no significant differences in the size of perch from L1 and L4. However, perch from these three lakes were significantly larger than those from L3, L5, and L6.

In L5, the piscivorous perch are significantly larger than the nonpiscivorous perch (i.e., trophic position <4) (piscivore mean body weight ± SE = 49.61 ± 3.01 g) versus nonpiscivore mean body weight = 30.00 ± 3.33 g) (*t* test, $F_{[1,38]} = 19.15$, $p < 0.0001$). However, piscivores from L5 are still significantly smaller than the piscivorous perch from L1 (trophic position ≥4, L1 mean body weight = 115.77 ± 8.58 g) (ANOVA and Tukey test, $F_{[2,31,0.05]} = 26.3$, $p < 0.001$). Similarly, in L6, the few perch that, according to their isotopic signatures, do become piscivorous (mean body weight = 45.91 ± 6.23 g) are significantly larger than other adult perch (mean body weight = 21.07 ± 2.17 g) that remain benthivorous (*t* test, $F_{[1,35]} = 14.16$, $p = 0.0006$). But the piscivorous perch in L6 are still considerably smaller than the piscivorous perch in L1 (one-way ANOVA and Tukey test, $F_{[2,31,0.05]} = 26.3$, $p < 0.001$). The mean body size of piscivorous perch was not significantly different in L5 and L6.

Finally, as prey size increased, so did the weight of perch (ANCOVA, $F_{[1,203,0.05]} = 41.74$, $p = 0.001$) (Table 5). However, the level of in situ benthic invertebrate diversity (indicated by H-group) and the interaction between in situ benthic invertebrate diversity and log(prey weight) were also significant, indicating that the size of prey is not independent of the level of diversity in the benthic community.

Fig. 3. Box plots of the body weights of adult yellow perch (*Perca flavescens*) (largest 25% of the population) from study lakes in Rouyn-Noranda. The central vertical line of the box represents the median of the sample, the length of the box represents the range within which 50% of the values fall, with the top and bottom of the box representing the 25th and 75th percentile of the data, respectively, and the hinges (vertical lines extending from the box) end at 1.5*x* (the range of the box). Lakes with the same letter are not statistically different from each other (one-way ANOVA followed by Tukey–Kramer multiple comparison test).



Benthic invertebrate diversity and abundance

Total benthic invertebrate diversity (H') was similar in L1–L4 (Table 6). The highly contaminated lakes (L5 and L6) had the lowest diversity (one-way ANOVA and Tukey–Kramer multiple comparison test, $F_{[5,79]} = 11.049$, $p = <0.0001$). One-way ANOVA and the Tukey–Kramer test comparing the mean invertebrate densities (number of individuals per square metre) of taxa among lakes revealed differences among lakes in amphipods, gastropods,

Table 5. Relationship between log(prey weight) and log(fish weight) with the level of benthic invertebrate diversity, represented by variable *H*, as a categorical variable for yellow perch (*Perca flavescens*) in Rouyn-Noranda lakes (n = 207).

Source	df	F	p
log(prey weight)	1	41.737	<0.001
H	1	24.252	<0.001
H × log(prey weight)	1	18.344	<0.001

hemipterans, hydrachnids, and trichopterans (Table 6). However, at the chosen level of α (p = 0.008), the differences were not significant in amphipods, dipterans, or hemipterans (Table 6). Although other taxa showed large decreases in mean density in contaminated lakes (e.g., Ephemeroptera and Odonata), the variation in the data within lakes was too large to detect significant differences.

Discussion

In metal-contaminated systems, it is clear that the physiologies of organisms can be impaired by the stress of coping with chronic exposure (Hontela et al. 1998). What is less clear is the contribution that indirect effects (i.e., effects mediated through the food web) have on animals at higher trophic levels. Although perch in Rouyn-Noranda are suffering from effects of direct exposure to metals (Laflamme et al. 2000; Campbell et al. 2003), changes in the in situ benthic invertebrate community offers an alternative explanation for some of the observed changes in the food webs of contaminated lakes.

In uncontaminated systems, juvenile perch generally progress from zooplanktivorous feeding to benthivory and often to piscivory. These ontogenetic switches are accompanied by increasing prey size. In Rouyn-Noranda, lakes with the lowest levels of contamination (L1 and L2) had diverse benthic communities and perch diets were characterized by high taxonomic richness. Adult perch in these lakes switched to piscivory or large benthic invertebrates like crayfish. The diverse benthic community in these lakes allowed perch to efficiently switch to larger prey items as they grew and as a result, perch attained normal adult body sizes.

In L1, all of the diet data are consistent with these progressive ontogenetic changes. The percent occurrence data show that perch switch from planktivory after age 1+ and then switch to piscivory at age 4+. The stable isotope data support this pattern, with increases in trophic position at similar ages. In L2, the perch diet data reflect fish that feed in an environment with abundant and diverse benthic resources. The percent occurrence data and isotopic evidence both suggest that perch do not switch to piscivory but remain benthivorous. However, the perch do switch to larger prey items like crayfish and odonates, as indicated by the prey size versus age relationship.

The perch in L3 and L4 have similar diets as the perch in L2 despite exposure to moderate levels of contaminants. There is no indication from the percent occurrence data or the stable isotope evidence that L3 and L4 perch switch to piscivory. A large proportion of the older age classes feed on

Table 6. Mean ± SE numerical density (no. invertebrates·m^{-2}) and Shannon's index (H') in Rouyn-Noranda lakes.

Lake	AMP	COL	DIP	EPH	GAS	HEM	ACA	ODO	BIV	TRI	H'
L1	21.2±12.2	1.1±0.6b	552.4±161.0	493.8±377.1	335.9±78.6abc	5.9±2.7	25.7±8.18	9.9±4.6	2493.7±1927.0	51.9±19.2bc	0.5±0.04a
L2	356.0±190.7	0.2±0.2b	1388.1±447.3	100.3±64.1	479.7±174.8a	0.48±0.48	92.6±19.9	8.75±6.74	1129.7±582.3	147.0±28.6ab	0.59±0.03a
L3	38.5±20.2	1.2±0.5a	326.1±98.4	54.9±15.4	220.6±57.0acd	3.4±1.2	10.9±3.7	6.2±2.2	31.4±8.2	28.6±5.5bc	0.6±0.04a
L4	134.7±58.9	0.0	511.2±146.9	60.03±20.6	240.7±79.3acd	0.9±0.8	25.8±7.4	15.1±8.0	118.8±49.4	52.5±18.1ab	0.6±0.05a
L5	3.3±2.3	5.7±2.0a	659.3±178.3	0.33±0.3	0.5±0.4cd	0.33±0.3	18.2±7.6	4.1±2.6	2491.7±1734.9	70.3±28.0abc	0.3±0.05b
L6	0.5±0.5	1.0±0.7a	261±54.2	39.3±23.5	0.5±0.5bcd	0.0	11.7±5.2	0.0	37.3±28.7	19.9±8.1bc	0.2±0.07b
p	0.009	**0.004**	0.018	0.481	**0.004**	0.034	**<0.0001**	0.385	0.645	**0.006**	**<0.0001**

Note: AMP, Amphipoda; COL, Coleoptera; DIP, Diptera larvae and pupae; EPH, Ephemeroptera; GAS, Gastropoda; HEM, Hemiptera; ACA, Acariformes (all Hydrachnidia); ODO, Odonata; BIV, Bivalvia (all Sphaeriidae); TRI, Trichoptera. n = 20, 15, 15, 15, 24, and 15 in L1–L6, respectively. Significant differences were established using one-way ANOVA (within taxa, among lakes) followed by a Tukey–Kramer test; p values in bold indicate significant differences (p < 0.008). Means followed by the same letter are not significantly different from each other.

large ephemeropterans (*Hexagenia*), odonates, and, in L4, crayfish. This is also reflected by the significant increase with age in the mean size of prey in their stomach contents.

In perch from L5 and L6, the ontogenetic patterns in diet differ from normal perch ontogeny. In lakes L1–L4, the isotope data are generally supported by the stomach contents data. This was not true for L5 and L6 perch. In L5, there has been no evidence of piscivory from stomach contents data in this study or from previous studies (Sherwood et al. 2000, 2002). The stable isotope data do indicate that most perch older than age 3+ have trophic positions of ~4, which is an indication of piscivory. Given that perch are the only fish in this lake, and those perch at a trophic position of 4 share the $\delta^{13}C$ of the age-1+ to age-2+ perch, it would appear that some of the older perch are cannibalizing the younger perch in L5.

In contrast with the diet patterns of perch from other lakes, a large proportion of older perch (>age 3+) in L6 still feed on zooplankton. This pattern has been observed in previous years (Sherwood et al. 2000), and hence, it is not just an anomaly of this study year. However, the stable isotope evidence indicates that L6 perch do rely on benthic invertebrates (trophic position of ~3 and $\delta^{13}C$ that is in the same range as benthos for that lake). It therefore seems that perch in L6 never fully switch out of a juvenile feeding stage. In uncontaminated systems, perch do not normally switch back to planktivorous feeding once they are older than age 2+ and have progressed to a different diet stage (Scott and Crossman 1973). They generally feed on larger, more energetically profitable prey. In L6, the fish community is still relatively diverse and includes several other littoral zone fish species such as suckers (*Catostomus* spp.) and northern pike. It is plausible that in L6, interactions with other fish species prevent perch from efficiently accessing the simplified benthic invertebrate community in the littoral zone. However, interactions with other fish species do not seem to prevent perch from accessing the more diverse benthic resources in L1 or L2.

The suitability of invertebrates as prey is partially related to their body size. The body size of prey items can alter the profitability of expending energy towards capture and consumption of those prey (Werner and Gilliam 1984; Sherwood et al. 2002). The size of prey in the stomach contents increases with age in L1–L4, which is to be expected from normal perch diet ontogeny. In the most contaminated lakes, perch do not shift towards large prey sizes as they age. This is supported by the observation that mean prey sizes in L5 and L6 are significantly smaller than the mean prey size in other lakes. This difference in prey size may have resulted from changes in the benthic communities of the different lakes, as the mean body size of in situ benthic invertebrates in L5 and L6 is smaller than that of invertebrates in L1 (Kövecses 2002). Therefore, in L5 and L6, perch are expending additional energy foraging for smaller prey than perch in L1, L2, or L4.

Although the stable isotope data indicate that some perch in L5 and L6 do become piscivorous, perch in these lakes do not seem to benefit energetically from this diet change to the same degree as perch in L1. In L5, the piscivorous perch are significantly larger than the nonpiscivorous perch (i.e., trophic position <4). However, the piscivores in L5 are still significantly smaller than the piscivorous perch from L1. Similarly, in L6, the few perch that, according to their isotopic signatures, do become piscivorous are significantly larger than other adult perch in the lake, but the piscivorous perch in L6 are still significantly smaller than the piscivorous perch in L1. It seems that perch in L5 and L6 do gain some energy advantage from piscivory, but it is not sufficient to achieve body sizes that are comparable with those of perch from systems with low levels of contamination. The energetic cost of handling larger prey can be higher then the cost associated with handling smaller prey. Hence, feeding on larger prey can result in the predator expending more energy on capture effort than it gains from the prey's larger body size (Lundvall et al. 1999). In L5 and L6, perch that have piscivorous isotope signatures are switching from consuming very small benthos to fish without the intermediate steps of consuming larger benthic invertebrates. The absence of larger benthic invertebrates in these lakes creates "holes" in the size distribution of available prey. This can ultimately contribute to inefficient predation and stunted fish.

Munkittrick and Dixon (1988) postulated that decreased growth of fish in metal-contaminated systems might be due to reduced total energy intake resulting from impoverished benthic invertebrate communities. Previous work on the bioenergetic performance of perch in Rouyn-Noranda lakes showed that perch in L3, L5, and L6 do not differ significantly from those in L1 or L2 in their consumption rates of prey. In these lakes, it is not a lack of total in situ invertebrate biomass that can explain the observed differences in adult perch body sizes among lakes — there are no significant differences between the contaminated lakes and L1 (Kövecses 2002). However, perch in L3, L5, and L6 do differ from perch in L1 and L2 in their conversion efficiencies (Sherwood et al. 2000) and in their activity costs, as measured by anaerobic enzyme activity in perch muscle tissue (activity was not measured in L3 or L4) (Sherwood et al. 2002). Furthermore, there are lower levels of benthic invertebrate diversity and the mean size of benthic invertebrates was smaller in L5 and L6 than in L1 (Kövecses 2002). These results imply that observed growth reductions in perch from contaminated lakes are due to the higher energetic costs of foraging and not simply a reflection of reduced energy intake.

The shift towards a less diverse prey base seems to have compromised the foraging efficiency of perch in contaminated lakes, which in turn has impacted the growth of perch. The results of the GLM (log(fish weight) versus log(prey weight), with benthic diversity level as a factor in the model) demonstrate that the ability of fish to shift to larger prey items is impacted by the level of benthic invertebrate diversity available in the community. The perch from L1, L2, and L4 attain large maximum body sizes, whereas the perch from the other lakes are stunted to various degrees (maximum size: L5 > L3 > L6). Perch from L5 and L6 exhibit stunted growth that would be expected from perch foraging in prey communities that have low diversity and low prey size availability. The same degree of stunting has been observed in wild yellow perch not exposed to metal contamination but lacking appropriate benthic prey sizes (Heath and Roff 1996). However, it is surprising that perch in L4 would be significantly larger than perch in L3, as there are no significant differences in perch from L3 and L4 in the bioaccumulation of metals in livers of the perch (Laflamme et al. 2000) and no differences in physiological stress responses, as measured by plasma cortisol levels (Campbell et al. 2003). It is unlikely that the factor or factors that are causing the differences in perch energetics in L3 and L4 are solely related to metal exposure, as the metal concentrations are higher in L4, yet perch are larger in L4 than those in L3. However, there are no significant differences in the diversity of the benthic invertebrate community between these two lakes, and although prey sizes tend to be larger in L4, the difference between L3 and L4 is not significant. Some other, as yet unknown, factor in the ecology of these lakes causes the observed impacts in yellow perch growth.

Any perturbation to a food web that weakens or removes energy pathways will affect higher trophic levels. For example, Boisclair and Rasmussen (1996) found that growth and consumption rates of yellow perch decreased in eutrophied lakes with the increased percent contribution of small prey in the invertebrate community. Additionally, Tyson and Knight (2001) found that before 1950, *Hexagenia*, trichopterans, and dipterans dominated the benthic community in Lake Erie. After dramatic increases in P loading, there was a shift to smaller bodied benthic invertebrates (dominated by chironomids). During this time, dipterans and zooplankton dominated adult perch diet and perch growth rates decreased. Water quality controls and the invasion of zebra mussels resulted in the return of larger benthic invertebrates and perch growth responded positively. This underscores the importance of access to large benthic invertebrates to the diet and growth of yellow perch.

The results from this study highlight the important role that ecological factors play in mitigating the impacts of exposure to metal contamination. In lakes with the lowest levels of contamination, the benthic community is diverse. This allows perch to shift to larger prey sizes, maintain feeding efficiency, and maximize their growth. In lakes with the highest levels of contamination, benthic communities were substantially less diverse and larger benthic invertebrate taxa were absent from the community and absent from the stomach contents of perch. The absence of larger benthic prey taxa potentially reduced the ability of feeding efficiently and as result contributed to the stunting of perch. If we are to effectively prevent further degradation of aquatic ecosystems and predict impacts and trajectories of recovery, we need to further our understanding of food web interactions and the ecology of contaminated systems.

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Modelling habitat requirement of European fishes: do species have similar responses to local and regional environmental constraints?

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Abstract: To test the hypothesis that different species have similar responses to local and regional environmental constraints, we modelled the occurrences of 13 species using a data set of 413 undisturbed river reaches. Three environmental descriptors were considered at the local scale (river slope, river width, and upstream drainage area) and three at the regional scale (mean annual and mean range air temperature and basin unit). Using multiple logistic regression modelling techniques, we correctly predicted the occurrence of 11 of the 13 retained species. The hierarchical partitioning analysis that we used allowed us to jointly consider all possible models in a multiple regression setting and to evaluate the independent explanatory power of each of our five environmental variables. We reject the hypothesis of a common species response to the environmental constraints. Species inhabiting upstream river reaches (bullhead (*Cottus gobio*), brown trout (*Salmo trutta*), minnow (*Phoxinus phoxinus*), and stone loach (*Barbatula barbatula*)) are more sensitive to basin unit. All species representative of downstream areas (barbel (*Barbus barbus*), dace (*Leuciscus leuciscus*), chub (*Leuciscus cephalus*), gudgeon (*Gobio gobio*), roach (*Rutilus rutilus*), bleak (*Alburnus alburnus*), and perch (*Perca fluviatilis*)) exhibit a positive continuous response to the drainage area, in agreement with the view of a continuous increase of local richness downstream. River slope is an important variable for all species. Main species habitat requirements are discussed for each species.

Résumé : À l'aide d'une banque de données couvrant 413 sections non perturbées de rivière, nous avons mis au point un modèle de la présence de 13 espèces afin de vérifier l'hypothèse selon laquelle les différentes espèces ont des réactions semblables aux contraintes environnementales locales et régionales. Nous avons tenu compte de trois variables descriptives du milieu à l'échelle locale (pente et largeur de la rivière, surface du bassin versant en amont) et de trois à l'échelle régionale (température moyenne annuelle de l'air, étendue moyenne des températures de l'air et unité du bassin versant). Des modèles de régression multiple logistique nous ont permis de prédire correctement la présence de 11 des 13 espèces choisies. L'analyse de classification hiérarchique utilisée nous a servi à considérer conjointement tous les modèles possibles dans un cadre de régression multiple et d'évaluer de façon indépendante le pouvoir explicatif de chacune des cinq variables environnementales. Nous rejetons l'hypothèse qui veut qu'il y ait une seule réaction commune des espèces aux contraintes du milieu. Les espèces qui habitent l'amont de la rivière (poisson-chat (*Cottus gobio*), truite brune (*Salmo trutta*), mené (*Phoxinus phoxinus*) et loche franche (*Barbatula barbatula*)) sont plus sensibles à l'unité du bassin. Toutes les espèces caractéristiques des zones d'aval (barbeau (*Barbus barbus*), dard (*Leuciscus leuciscus*), chevaine (*Leuciscus cephalus*), goujon (*Gobio gobio*), gardon (*Rutilus rutilus*), ablette (*Alburnus alburnus*) et perche (*Perca fluviatilis*)) montrent un continuum de réactions positives à la surface du bassin versant, en accord avec l'argument qui veut qu'il y ait une accroissement continu de la richesse locale vers l'aval. La pente de la rivière est une variable importante pour toutes les espèces. Les besoins principaux d'habitat de chaque espèce font l'objet d'une discussion.

[Traduit par la Rédaction]

Introduction

Knowledge of species distribution and the capacity to predict the occurrence of fish species are crucial to managing aquatic biodiversity and to assessing the consequences of anthropogenic changes in the river environment, such as global climate change (Ormerod 2003). Until recently, most modelling efforts were centered on building predictive tools describing fish species responses to local environmental conditions, with a particular emphasis on hydraulic variables

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(Bovee 1982; Gore and Nestler 1988). Most of these studies only apply to one particular river in a specific region and thus cannot be directly transferred to other basins belonging to geographically distant regions (Leftwich et al. 1997). More recently, some authors have developed significant models using data pooled from different rivers (Lamouroux et al. 1999) but did not include potentially important large-scale variables such as temperature.

It is now a common view that local species occurrences are not only constrained by local processes (e.g., physical constraints, habitat diversity, and biotic interactions) but also by factors operating at larger temporal and spatial scales, such as climate, dispersal barriers, and historical biogeography (Tonn et al. 1990; Huston 1999). The general hypothesis is that large-scale processes determine the pool of species available to occur locally, whereas small-scale processes tend to limit the number of species that actually occur at a given site. Therefore, modelling the occurrence of fish species in local assemblages should also account for the influence of regional factors that condition the presence of species within each drainage unit (Oberdorff et al. 2001).

Previous works clearly identified local variables, as well as large-scale features, that could influence fish species distribution (Matthews 1998). As fish species cannot disperse across terrestrial areas, drainage basins represent ecologically relevant regions for these organisms (Matthews 1998) and should be considered as a main regional factor with regard to long-term dispersal processes. In addition, drainage basins are natural units linking various components of terrestrial and aquatic ecosystems (e.g., soils and waters and productivity) making them, in essence, more or less unique (Hornung and Reynolds 1995). Thermal climate is also an important determinant of fish species distributions at a regional scale acting on the spawning period or egg development and mortality (Crisp 1996; Mann 1996). Rahel and Hubert (1991) considered that, within a basin, temperature operates at a relatively large scale and that changes in thermal conditions can explain replacements of one fish assemblage by another. Variation in fish assemblage composition along the upstream–downstream gradient of rivers is a widely studied aspect of fish ecology. These longitudinal changes are usually attributed to one of two processes: biotic zonation or continual addition of species downstream in association with the increase in river size and habitat diversity (Huet 1959; Schlosser 1982; Pont et al. 1995). At the local scale, hydraulic conditions and habitat diversity are known to exert a strong influence on fish communities (Horwitz 1978; Matthews 1998). Variables describing the general morphology of a river reach (river slope and river width) and mean hydraulic conditions (Lamouroux et al. 1999) can summarize the most important environmental features at that local scale (reach scale).

Comparatively little is known about the relative importance of these local and regional factors and their influence on the occurrence of each species of an assemblage. Recent studies dealing with interactive effects of local and regional factors only considered the community level, mainly species richness (Tonn et al. 1990; Angermeier and Winston 1998), or synthetic descriptors of community structure (Jackson and Harvey 1989). Here, all species are implicitly considered to have the same response to the hierarchical organization of their environment. Although assemblage-level analysis is important in detecting overall patterns or testing general hypotheses, the detailed analysis of individual species may provide a better understanding of the mechanisms or factors in play behind observed patterns of species occurrence. As proposed by Kolasa (1989), all species do not have the same perception of the heterogeneity of their habitat. As in the case of species richness, understanding the occurrence patterns of a given species in local assemblages requires considering the influences of regional factors that affect the presence of species within each drainage unit (Matthews 1998). Examining the influence of these environmental factors on the occurrence of each species separately may also serve to test the hypothesis of a common response of each fish species of a local assemblage to their local and regional environment.

In this paper, using a site-specific approach, we present habitat-based statistical models for predicting the occurrence of the most common riverine fish species in France. Our study includes river reaches belonging to the four largest French river basins, which represent a significant part of the whole geographical range of the species considered. Our detailed objectives are (*i*) to evaluate the efficiency of our six environmental variables to accurately predict species presence–absence, (*ii*) to compare species responses to environmental constraints and to discuss the balance between local and regional factors, and (*iii*) to, after selecting, for each species, the environmental variables having the higher independent explanatory power, describe species habitat requirements.

To evaluate the independent explanatory power of each environmental variable, we used hierarchical partitioning (Chevan and Sutherland 1991), a method in which all possible models in a multiple regression setting are considered jointly to attempt to identify the most likely causal factors.

Materials and methods

Data set

We extracted a data set of river reaches from the database of the National French Fishing Council (1985–1998 period) base on three criteria. Initially, reference sites were selected on the basis of classic water quality criteria and of a limited degree of river channel modifications (Oberdorff et al. 2001). These reference sites were not totally undisturbed, but rather those sites that have suffered comparatively limited human impact within a particular basin. We also retained exclusively wadeable sites (*i.e.*, mean river depth not over 1 m) where fishes were sampled by electrofishing with one pass during low-flow periods (August–October).

Lastly, as it is well known that the species richness within a given river basin is highly correlated with the size of that basin (Welcomme 1979), the mean occurrence of a species at regional scales should also depend on basin size. To avoid the influence of basin size on the expected occurrence of a species within a given river reach, we only retained basins of similar size (Loire River, 113 812 km^2; Rhône River, 97 800 km^2; Garonne River, 84 811 km^2; Seine River, 73 700 km^2) (Fig. 1). The distributions of our environmental variables are quite comparable among these basins (Table 1), but they do still differ by their climate (Oceanic to Mediterranean) and geological characteristics.

Fig. 1. Map of France showing the four studied basins and the 413 references sites.

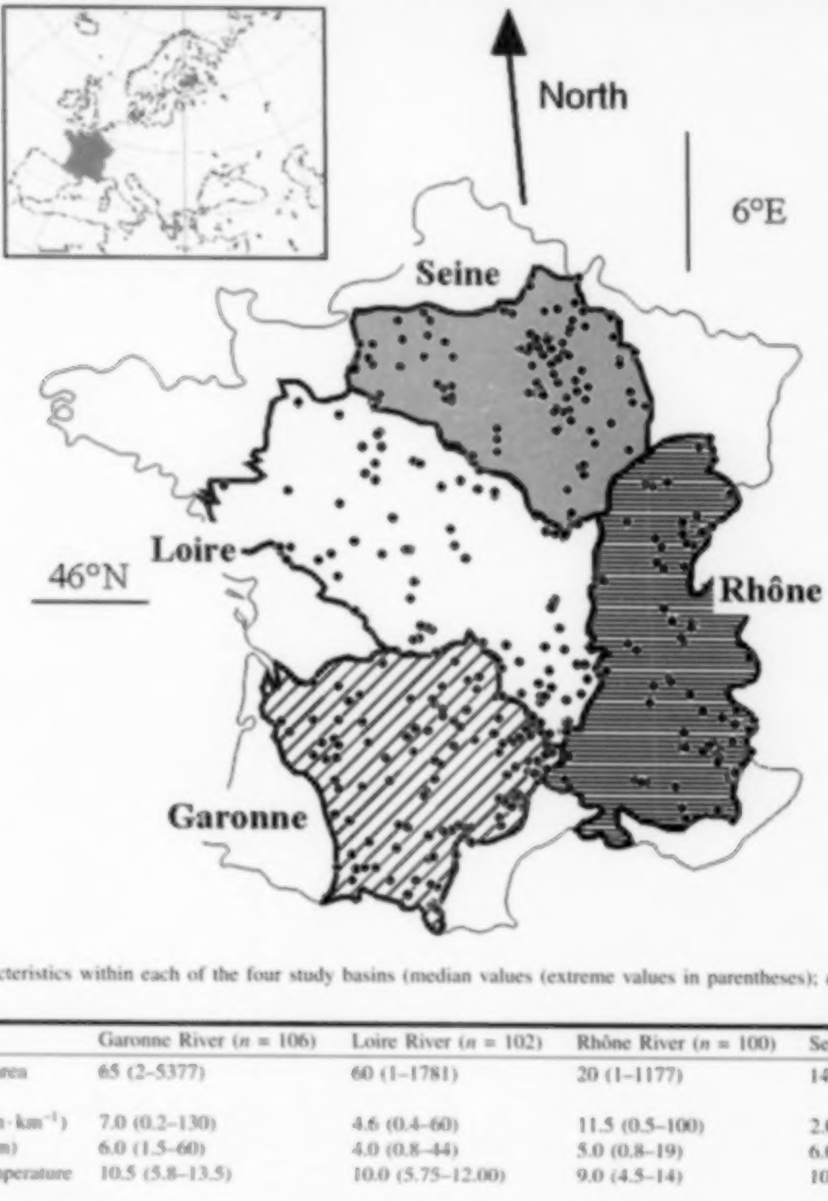


Table 1. Sites characteristics within each of the four study basins (median values (extreme values in parentheses); *n* is the number of sites per basin).

Variable	Garonne River (n = 106)	Loire River (n = 102)	Rhône River (n = 100)	Seine River (n = 105)
Upstream drainage area (UDA, km^2)	65 (2–5377)	60 (1–1781)	20 (1–1177)	146 (2–9233)
River slope (SLO, m·km^{-1})	7.0 (0.2–130)	4.6 (0.4–60)	11.5 (0.5–100)	2.0 (0.5–12)
River width (WID, m)	6.0 (1.5–60)	4.0 (0.8–44)	5.0 (0.8–19)	6.0 (1.0–60)
Mean annual air temperature (MAT, °C)	10.5 (5.8–13.5)	10.0 (5.75–12.00)	9.0 (4.5–14)	10.1 (8.8–10.5)
Mean annual air temperature range (MAR, °C)	15 (11–21)	15 (13–18)	17 (13.5–19.5)	16 (12.5–17)

The 13 species retained are among the most common autochthonous species in France. They have a mean occurrence of at least 10% in our data set (Table 2) and are all present over all four basins in the study. This avoids any effect due to overly restricted distribution of a species. Most of these species are cyprinids: bleak (*Alburnus alburnus*), barbel (*Barbus barbus*), gudgeon (*Gobio gobio*), chub (*Leuciscus cephalus*), dace (*Leuciscus leuciscus*), minnow (*Phoxinus phoxinus*), and roach (*Rutilus rutilus*). The six others species are stone loach (*Barbatula barbatula*), northern pike (*Esox lucius*), brown trout (*Salmo trutta*), perch (*Perca fluviatilis*), bullhead (*Cottus gobio*), and brook lamprey (*Lampetra planeri*).

Environmental variables

The National French Fishing Council database includes several environmental descriptors at the reach level. For our

Table 2. Mean species occurrences.

Species	Species occurrence	Kappa	% of sites correctly predicted: All sites	Sites with fish	Sites without fish
Barbel	0.174	0.584	88.6	61.1	94.4
Bleak	0.126	0.613	91.8	63.5	95.8
Brook lamprey	0.194	0.101	79.7	11.2	96.1
Brown trout	0.852	0.473	88.6	96.3	44.3
Bullhead	0.554	0.404	70.5	72.1	68.5
Chub	0.378	0.571	79.9	72.4	84.4
Dace	0.223	0.538	84.5	60.9	91.3
Gudgeon	0.455	0.501	75.3	71.8	78.2
Minnow	0.632	0.465	76.8	90.8	52.6
Perch	0.179	0.419	85.7	39.2	95.9
Northern pike	0.121	0.220	87.4	20.0	96.7
Roach	0.293	0.540	81.6	62.8	89.4
Stone loach	0.622	0.566	80.4	90.3	64.1

Note: Classification tables for the multiple logistic regression models to estimate the probability of the presence of the 13 species within the 413 French sites. Models include all six environmental variables. Shown are values of the Kappa statistic and the percentages of all 413 sites correctly predicted with or without fish, sites with fish correctly predicted with fish, and sites without fish correctly predicted without fish.

purpose, we retained six descriptors that can directly influence the occurrence of fish species.

The river slope (SLO, $m \cdot km^{-1}$) is a determinant factor for hydraulic conditions at the reach scale, as demonstrated by the classic general Chezy formula (Gordon et al. 1992), and is known to be one of the main factors influencing fish species distribution (Huet 1959). River width (WID, metres, measured at the sampling date) is indicative of both river size and habitat diversity at the reach scale (Matthews 1998). The upstream drainage area (UDA, square kilometres) was used to describe the position of a given reach within the overall drainage basin.

As streams and rivers are relatively shallow, turbulent, and well-mixed systems, they easily exchange heat with the atmosphere. Consequently, mean annual air temperature (MAT, degrees Celsius) and mean annual air temperature range (MAR, degrees Celsius) were used as a surrogate of water temperature to describe thermal conditions within each river reach. The baseline climate data set was generated from mean July and January monthly values (1951–1993 period) for all official French weather stations, interpolated and mapped by the French Meteorological Service.

SLO, WID, and UDA are good descriptors of physical and hydrological conditions at the local scale (Oberdorff et al. 2001). MAT and MAR are representative of climatic conditions at the reach scale but are considered to be large-scale descriptors because of their high level of spatial autocorrelation at a regional scale. Finally, the basin unit (BAS) is for fish a good synthetic descriptor of regional environmental constraints and is retained as a dummy variable (Loire: LOIR, Rhône: RHO, Garonne: GAR, Seine: SEI).

SLO, WID, and UDA were log-transformed to reduce the skewness of their distribution. MAT and MAR were nearly normally distributed and were thus kept untransformed in our analyses. The number of sites per basin (Fig. 1) was almost identical (from 100 to 106 sites).

Logistic modelling method

When using logistic regression methods, the "presence–absence response curve" of a species (ter Braak and Looman 1986) describes the probability of the species being present, p, as a function of a measured environmental variable, x. The general expression for this probability is

$$(1) \qquad \log\left(\frac{p(x)}{1 - p(x)}\right) = f(x) \Leftrightarrow p(x) = \frac{e^{f(x)}}{1 + e^{f(x)}}$$

where $f(x)$ is a linear combination of variable x. Equation 1 can be extended to include more than one explanatory variable to which multiple logistic regressions can be applied. The contribution of each variable of the linear combination $f(x)$ in a logistic regression model is assessed by comparing the predictive value of models, with and without the variable in question, using the likelihood ratio test based on the difference of the deviance ($D = -2$ log(likelihood ratio)) between the two models. The significance of reduction in deviance is tested by comparing the observed value with a χ^2 distribution (G test, S-PLUS procedure (MathSoft Inc., Seattle, Washington)).

Modelling efficiency

To evaluate the efficiency of our six environmental variables to accurately predict species presence–absence, we built multiple logistic regression models integrating all of the variables and their quadratic terms. As BAS was a dummy variable, we only considered the first-order model. The complete model is

$$(2) \qquad f(x) = f(\mathrm{SLO}, \mathrm{SLO}^2, \mathrm{WID}, \mathrm{WID}^2, \mathrm{MAR}, \mathrm{MAR}^2, \mathrm{MAT}, \mathrm{MAT}^2, \mathrm{UDA}, \mathrm{UDA}^2, \mathrm{BAS})$$

A cross-validation procedure was used to judge the model's efficiency. Each of the models was fitted by using all but one of the sites, and this procedure was repeated by omitting each of all of the 413 sites. The presence–absence of each species for a site was predicted by rounding off each species' expected probability to 0 or 1 (predicted probability lower or higher than 0.5) (Colett 1999). The performances of the 13 models were summarized in a confusion matrix that cross-tabulates the observed and predicted presence–absence patterns (Fielding and Bell 1997) and identifies the two possible prediction successes (true positives and true negatives) and the two possible prediction errors (false positives and false negatives). Manel et al. (2001) evaluated several measures of the performance of logistic models and recommended Cohen's Kappa index. This widely used index assesses the extent to which models correctly predict occurrence at rates that are better than would be expected by chance (Fielding and Bell 1997). Values of 0.0–0.4 are considered to indicate poor to fair model performance. Therefore, we only retained models having a Kappa index ranging between 0.4 and 1.

Species responses to the hierarchical organization of their environment

Multivariate regression methods are a common way of determining the relative importance of the effect of N independent variables (Xs) upon a dependent one (Y) and to make inferences about the determinants affecting species patterns. Recently, McNally (2000) pointed out that model selection in the multiple regression context has two distinct purposes. The first is to produce the best single and parsimonious model to predict values of the dependent variable (predictive approach), and stepwise selection of a subset of independent variables having a significant effect on the dependent one is the most widespread technique. The second approach involves using the outcomes of the different possible models to infer the relative influence of X variables upon Y variables, exploring for potentially causal or explanatory relationships (explanatory approach). The search for a single best model has little to do with explanation because the fact that the regression minimizes the overall difference between the observed and predicted Y values does not necessarily equate to determining probable influence in a multivariate setting. Single-model multivariate approaches cannot identify situations in which potentially important independent variables are suppressed by other independent variables. And this problem increases with the number of independent variables and with the colinearity in Xs. When there is colinearity between predictors, the direct response of a species to a descriptor may in fact only be an indirect effect owing to high dependence of the considered descriptor with one or many other descriptors. In our data, the six environmental descriptors were not independent, even though we avoided descriptors that were too highly correlated with others. Pearson's coefficient values ranged from –0.558 to +0.712.

Another approach is to use hierarchical partitioning (Chevan and Sutherland 1991), a method based on the theorem of hierarchies in which all possible models in a multiple regression setting are jointly considered to attempt to identify the most likely causal factors. If we consider N, the number of explanatory variables (X_1, X_i, X_N), there are 2^N possible models, including the null model (M_0, M_{x_1}, …, $M_{x_{123\ldots N}}$). The R_i is a measure of fit between one independent variable X_i and the dependent variable Y. As we used logistic regression to treat our presence–absence data, the measure of fit between each of our six explanatory variables and our dependent variable Y (species occurrence) is the reduction of deviance generated by introducing a given variable into all of the possible models built with the five other variables within the considered hierarchies.

Chevan and Sutherland (1991) demonstrated that we have to consider $N!$ hierarchical orderings of models that always begin with M_0 and end with $M_{x_{123\ldots N}}$. For example, with $N = 3$, the six hierarchical ordering will be as follows:

1	2	3	4	5	6
M_0	M_0	M_0	M_0	M_0	M_0
M_{x_1}	M_{x_1}	M_{x_2}	M_{x_2}	M_{x_3}	M_{x_3}
$M_{x_{12}}$	$M_{x_{13}}$	$M_{x_{21}}$	$M_{x_{23}}$	$M_{x_{31}}$	$M_{x_{32}}$
$M_{x_{123}}$	$M_{x_{123}}$	$M_{x_{123}}$	$M_{x_{123}}$	$M_{x_{123}}$	$M_{x_{123}}$

For any given initial variable X_i, there are $(N - 1)!$ possible hierarchies containing $N(N - 1)!$ models in which X_i appears. For each hierarchy, one could evaluate the influence of X_i on each of the N models including X_i (increase in model fit generated by including the variable X_i within each model). The independent influence of X_i on Y (I_i) will be obtained by averaging all of the $N(N - 1)!$ increases of fit. This averaging is likely to alleviate multicolinearity problems that are ignored by using any one-model technique.

The joint component J_i (effect caused jointly with the $N - 1$ other variables) is obtained by subtracting I_i from R_i, with $R_i = I_i + J_i$. Chevan and Sutherland (1991) recommended focusing on variability accounted for by a variable (independent effect I_i) and expressing the impact of the variable in percentage terms ($\%I_i$), with $\%I_i = \dfrac{I_i \times 100}{R_i}$. We considered that, for a given species, a variable would have a high independent explanatory power when $\%I_i$ was higher than the mean value, i.e., 100/6 = 16.6%.

When considering all of the independent (I) and jointed (J) effects of the N variables, R is given by

$$R = I + J = \sum_{i=1}^{n} R_i = \sum_{i=1}^{n} I_i + \sum_{i=1}^{n} J_i$$

If all explanatory variables were completely independent of one another, there would be no joint effects (Chevan and Sutherland 1991). Therefore, the difference between R and I reflects the degree to which the variables, taken as a whole, are acting jointly. McNally (2000) recommended looking not only at the independent component of the measure of association but also at the ratio of I over J. A ratio below 1 would indicate that the variables appear to have complex interrelationships.

Table 3. Decomposition of the total reduction in deviance (*R*) associated with the six environmental variables into independent (*I*) and dependent(*J*) components of *R* using the hierarchical partitioning method.

	Total partition				$\%I_i$					
Species	*R*	*I*	*J*	*I*/*J*	SLO	WID	UDA	MAR	MAT	BAS
Bullhead	−217	−130	−86.5	1.51	16.8	3.3	7.1	2.5	28.1	42.2
Brown trout	−294	−157	−138	1.14	26.9	5.1	15.9	8	20.1	23.9
Minnow	−225	−151	−73.7	2.05	47.5	5.6	8.8	4	5.9	28.3
Stone loach	−334	−202	−131	1.54	49.5	4.1	11.7	7	7	20.7
Dace	−449	−197	−252	0.78	24.1	28.1	25.1	1.5	10.5	10.8
Barbel	−404	−196	−208	0.94	19.9	27.3	32.1	5.7	9.2	5.9
Chub	−460	−209	−251	0.83	32	16.2	23	4.1	17.9	6.8
Gudgeon	−411	−192	−219	0.88	27.2	13.7	24.8	3.7	17	13.7
Roach	−439	−198	−241	0.82	38.9	5.9	19.5	1.4	22.5	11.8
Bleak	−389	−185	−204	0.9	20.1	13.5	34.7	3.2	19.8	8.7
Perch	−224	−111	−113	0.99	42	9	25.6	2.2	6.1	15.1
Mean effect					31.4	12	20.8	3.9	14.9	17

Note: Independent effect ($\%I_i$) associated with each environmental variable and expressed as a percentage of the total independent effect *I* associated with the variable considered. Species are gathered into five types according to their sensibility for each of the five environmental variables. Mean $\%I_i$ values when considering the 11 species. SLO, river slope; WID, river width; UDA, upstream drainage area; MAR, mean annual air temperature range; MAT, mean annual air temperature; BAS, basin unit.

Species habitat requirements

For each species, we retained variables for which the relative independent effects were the most important, and we performed analyses to investigate the ecological responses.

To evaluate the ecological responses of the selected environmental variables, we tested the species responses to one of the environmental variables (SLO, for example) after considering the effect of all of the five others by pairwise comparison of the following models:

$$(3)\quad f(x) = f(\mathrm{MAR}, \mathrm{MAR}^2, \mathrm{MAT}, \mathrm{MAT}^2, \mathrm{WID}, \mathrm{WID}^2, \mathrm{UDA}, \mathrm{UDA}^2, \mathrm{BAS})$$

$$(4)\quad f(x) = f(\mathrm{MAR}, \mathrm{MAR}^2, \mathrm{MAT}, \mathrm{MAT}^2, \mathrm{WID}, \mathrm{WID}^2, \mathrm{UDA}, \mathrm{UDA}^2, \mathrm{BAS}, \mathrm{SLO})$$

$$(5)\quad f(x) = f(\mathrm{MAR}, \mathrm{MAR}^2, \mathrm{MAT}, \mathrm{MAT}^2, \mathrm{WID}, \mathrm{WID}^2, \mathrm{UDA}, \mathrm{UDA}^2, \mathrm{BAS}, \mathrm{SLO}, \mathrm{SLO}^2)$$

We tested the significance of the reduction of deviance between pairs of models (*G* test using a χ^2 distribution, S-PLUS procedure (MathSoft Inc.)). The response of the species to SLO could be insignificant (null model, eq. 3) or significant with a continuous increase or decrease (sigmoid response, eq.4) or characterized by a symmetrical and bell-shaped response curve (eq. 5) (ter Braak and Looman 1986). As recommended by Venables and Ripley (1999), when a second-order form of a variable is retained, the first-order form of this variable is systematically added to the model. As the BAS variable was a dummy variable, we did not consider the second-order model.

To demonstrate the ecological significance of our models in terms of habitat requirements, we defined their marginal profile for each habitat characteristic. For each species and for a given local variable, we computed the predicted probability of species presence all along the observed variable range by using our previous logistic model, while all other environmental variables were held constant at their median values. Then for each species and each environmental variable, we defined an optimal condition corresponding to the highest probability of occurrence of the considered species ($P_{\max}$). For the BAS variable, we evaluated the effect of each of the four basins by examining the regression coefficients associated with each of them.

All statistics were computed using S-PLUS 6 software (MathSoft Inc.) and following the recommendations of Venables and Ripley (1999).

Results

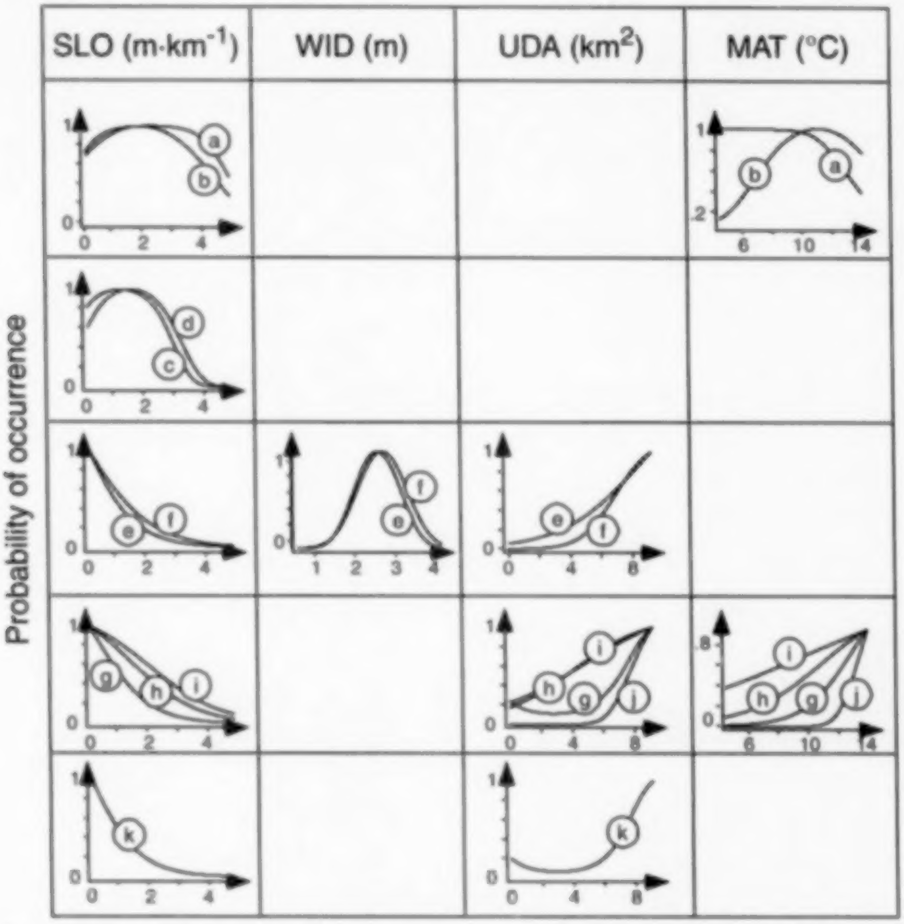
Data set characteristics

Our data set covered a wide range of environmental conditions within the four basins (Table 1), but it did not include very large river sections (WID up to 60 m and UDA up to 9233 km^2). Species frequencies (Table 2) ranged from 0.121 (northern pike) to 0.852 (brown trout). The species studied are well represented within the four basins, except for bleak and northern pike in the Rhône basin (0.01 and 0.03, respectively).

Modelling efficiency

According to our validations, the species-specific multiple logistic regression models with the six environmental variables correctly predicted species presence and species absence for 71–92% of the sampled sites, depending on the

Fig. 2. Specific marginal effect of each environmental variable (SLO, river slope; WID, river width; UDA, upstream drainage area; MAT, mean annual air temperature) conditionally to a fixed value of the other variables (i.e., their median value) when the variable showed a high independent explanatory power. SLO, WID, and UDA are log-transformed. The probability of occurrence of a species is expressed in relation to its maximal probability of occurrence for SLO, WID, UDA, and MAT. Curves: *a*, brown trout (*Salmo trutta*); *b*, bullhead (*Cottus gobio*); *c*, minnow (*Phoxinus phoxinus*); *d*, stone loach (*Barbatula barbatula*); *e*, barbel (*Barbus barbus*); *f*, dace (*Leuciscus leuciscus*); *g*, roach (*Rutilus rutilus*); *h*, chub (*Leuciscus cephalus*); *i*, gudgeon (*Gobio gobio*); *j*, bleak (*Alburnus alburnus*); *k*, perch (*Perca fluviatilis*).



species (Table 2). As expected (Manel et al. 2001), the percentage of true positive and true negative sites in our confusion matrix was not independent of the species prevalence. Kappa values ranged from 0.42 to 0.61 for 11 species. Two species (northern pike and brook lamprey) were associated with very low Kappa values (0.22 and 0.10, respectively) and their models were not retained.

Species responses to the hierarchical organization of their environment

Four of the 11 retained species (bullhead, brown trout, minnow, and stone loach) had total independent contributions (I) much larger than their joint effects (J) (Table 3), with the I/J ratio ranging from 1.14 to 2.05. SLO account for more than 17% of the total independent effect for all species and with a mean $\%I_i$ value of 31.4%. This local variable is of primary importance for stone loach ($\%I_i$ = 50%), minnow (48%), perch (42%), and roach (39%). More than 19% of the total independent variability was accounted for by UDA when considering barbel, bleak, dace, gudgeon, perch, chub, and roach. But UDA was the most important variable only for barbel (32%) and bleak (35%). Six species showed a strong independent effect of MAT (range 17–28%): bullhead, brown trout, chub, gudgeon, roach, and bleak. MAR never appeared as having a important independent effect for the studied species, and WID only gave strong independent effects for dace and barbel. BAS was the main explanatory variable for bullhead ($\%I_i$ = 42%) and also accounted for a significant part of the total independent contribution of the six environmental descriptors for minnow (28%), brown trout (24%), and stone loach (21%).

Five types of species responses were distinguished based on which variables showed high explanatory contributions in the models. Bullhead and brown trout were characterized by the explanatory power of BAS, MAT, and SLO. BAS and SLO were of first importance for minnow and stone loach. The three local variables (SLO, WID, and UDA) were retained for dace and barbel but only two (SLO and UDA) for perch. The remaining species (chub, gudgeon, roach, and bleak) were characterized by SLO, UDA, and MAT. The cumulated independent effect of the three regional environmental variables (MAT, MAR, and BAS) explained respectively more than 50% of the total explanatory power of the six considered variables for bullhead (73%) and brown trout (52%) versus 21–38% for the other nine species.

Species habitat requirements

The multiple regression models obtained by adding, when significant, a given variable (in first- or second-order form) to a previous model including the five other variables demonstrated the sensitivity of the species to the selected variables (Fig. 2) and described the type of responses (sigmoidal versus bell-shaped).

All species, exception for bleak, were sensitive to SLO, with p values ranging from 0.032 to 1×10^{-7}. Four of the response models included a second-order term (bullhead, brown trout, stone loach, and minnow). The optimal slope ranged from 8.87‰ for brown trout to 6.17‰ for bullhead, 4.00‰ for minnow, 2.67‰ for stone loach, and 0.20‰ for others species. Among the five species having their ecological optima at the lowest SLO condition, roach and perch appeared to have more restricted ranges. Brown trout demonstrated a particular response curve to SLO with a very wide range and a decrease in its its probabilities of occurrence only at the two extremities of the slope. WID had a significant effect on the occurrences of dace and barbel, which both demonstrated a clear optimum for intermediate WID values (9.8 and 13.4 m, respectively).

A significant effect of UDA was noted for seven species ($1.8 \times 10^{-2} < p < 3.6 \times 10^{-7}$). All exhibit a continuous positive response to UDA with a common optimum for the highest value of the gradient (9233 km^2). However, roach, perch, and especially bleak appeared to be more restricted to the downstream river reaches than gudgeon, chub, and dace.

The predicted probability of occurrence for brown trout remained close to its maximum value throughout most of the thermal gradient, but with a avoidance of MAT values over 12 °C. Bullhead preferred intermediate thermal conditions, with an optimum at 11 °C. The four other species demonstrated a continuous positive response. Roach and especially bleak were more restricted to the warmest river reaches in comparison with gudgeon and chub.

At the regional level, BAS had a significant effect for only four species (trout, bullhead, minnow, and stone loach). All of these species exhibited a positive response for the Loire basin (regression coefficients of 0.484, 1.283, 1.537, and 1.665, respectively). The Seine River was also preferred by bullhead (2.356) and stone loach (1.139). The two others basins (Garonne and Rhône rivers) were never clearly preferred.

Discussion

The present study was designed to investigate riverine fish species responses to environmental constraints by addressing three main targets: (*i*) evaluating the global efficiency of our modelling approach, (*ii*) comparing species responses to environmental constraints and discussing the balance between local and regional factors in species occurrence patterns, and (*iii*) describing species habitat requirements.

The multiple logistic regression models correctly predicted the incidence for 11 of the 13 studied species. For northern pike and brook lamprey, the Kappa index was lower than 0.4 and we only correctly predicted species absence, probably because of their specific habitat requirements (Philippart and Vranken 1983), which were not considered in our approach. Northern pike usually leave the main channel to reproduce in backwaters with vegetation, and brook lamprey spend most of their life in the larval stage buried in substrate.

Our second objective was to test the relative importance of local and regional environmental constraints. The hierarchical partitioning analysis that we used (Chevan and Sutherland 1991) allowed us to jointly consider all possible models in a multiple regression setting to attempt to identify the most likely causal factor (McNally 2000). The first outcome of our hierarchical partitioning analysis is that some of our six environmental variables have a substantially greater independent explanatory power than others. By testing the assumption that the six variables had equivalent explanatory power (16.7%), slope was found to have an explanatory power higher than expected for all 11 species. At the reach scale, slope, as a surrogate of mean local hydraulic conditions, is a determinant factor of fish species occurrences (Huet 1959; Lamouroux et al. 1999). Longitudinal position is an important factor but not the dominant one, except for barbell and bleak. MAT is of primary importance for brown trout and bullhead, two cold-water species, and gudgeon, chub, roach, and bleak, which prefer the warmest waters. For these species, MAT may be a key variable in explaining their geographic range.

One of the more interesting results is that factors that operate at a regional scale (temperature and basin unit) have the highest independent explanatory power for the two species inhabiting the smallest streams (brown trout and bullhead). In addition, the basin scale effect is of major importance for the four species inhabiting the upper river reaches (brown trout, bullhead, stone loach, and minnow). And only these five species have a total independent effect larger than their sums of joint effects. Our results clearly demonstrated than the responses of the typical rhitronic fish fauna to their environmental constraints differed from those of species inhabiting larger rivers.

Angermeier and Winston (1998) examined the relative influence of local versus regional factors on the local diversity of fish communities inhabiting third- to fifth-order streams, and they also found regional predictors to outweigh local predictors in their multiple regression models. Our result could be due to the greater sensitivity of rhitronic species to temperature but also to a more pronounced variability between basins among upstream river reaches. Differences between physical and chemical characteristics of a set of rivers tend to increase when going upstream in relation to the local influences of the different drainage areas and their associated geological and geomorphologic characteristics. However, one could also argue that species tolerance to environmental constraints may vary between basins in relation to genetic variability among populations. The bullhead, which appears as highly sensitive to basin effect, is now known to show a high level of genetic differentiation among populations. Volckaert et al. (2002) demonstrated the presence of different clades corresponding to locations in the Loire, Garonne, Seine, and Rhône basins. Evidence of genetic differentiation has also been documented for brown trout (Guyomard 1991) and minnow (Mueller and Ward 1998).

Furthermore, the contrasting sensitivity of the 11 selected species to the six environmental descriptors leads us to reject the hypothesis that species of site assemblages have common responses to the hierarchical nature of their environment. Our study confirms that the sensitivity to local and regional scale processes are species specific, and as proposed by Kolasa (1989), all species do not have the same perception of the heterogeneity of their habitat. Our results also support the concept of environmental filters and the idea that a biologically based approach to predict species distribution needs to explicitly consider environmental constraints imposed at different spatial scales (Tonn et al. 1990). Two categories of processes operating at large scales should be distinguished (Huston 1999): (*i*) evolutionary processes that really operate at large scales (species migrations, regional extinction, and speciation) and (*ii*) ecological patterns that mostly appear at large scales but that actually operate or can be measured at local scales, such as geophysical or climatic properties of a region. These two kinds of processes, here accounted for by the influence of temperature and basin unit on species occurrence, are both observed in our data.

Further work is needed to better identify the processes underlying these effects on species distribution, and different interpretations or directions of investigation should be developed. According to metapopulation theory, the probability of presence of a species in a patch depends on local features of the site but also on regional factors such as the number of potential colonization sources and the connectedness of the focal patch to these sources. A few studies suggest that, within a basin, between-patch dispersal cannot be neglected when considering local fish community structure (Osborne and Wiley 1992; Grenouillet et al. 2004). The fragmentation of aquatic habitats may be greatly increased by channelization and dams, and in this regard, the Loire and Seine basins are moderately affected, whereas the Rhône and Garonne basins were noted as strongly affected (Nilsson and Dynesius 1994). And it is well recognized that the Loire basin is the least impacted among the large French basins. In accordance with this observation, we demonstrated that the Loire basin has a positive effect for all four rhitronic species.

For the environmental variables considered, the independent explanatory power is determined in relation to each species. The quality of our results can be evaluated by comparing the habitat requirements determined by our models with current available knowledge. On the whole, hierarchical partitioning analysis allowed us to distinguish five types of species response in which the species of type showed similar habitat requirements, i.e., similar marginal profiles to the selected environmental variables.

SLO is one of the main factors controlling water velocity (Gordon et al. 1992). At the reach scale, slope exerts of stream physical habitat structure in association with stream size (Hubert and Kozel 1993). Our results highlight the major importance of slope for almost all of the considered species and fit well with current knowledge (Philippart and Vranken 1983; Crisp 1996; Mann 1996). Species ordinate according to SLO from slow- to fast-flowing water. Lamouroux et al. (1999) also analysed the habitat requirements of fish species in relation to water velocity, depth, and roughness. They described barbel as having a positive response to water velocity similar to that of bullhead, whereas brown trout preferred lower velocity. In our opinion, the differences between these observations and our results are primarily related to a scale effect. Lamouroux et al. (1999) described fish habitat preference at the microhabitat scale, whereas we focus at the reach scale. The responses of the studied species to slope at the reach scale could be considered as a combination of responses in relation to the anatomical adaptation of species and their swimming capacity and to the presence of physiological adaptations to low dissolved oxygen levels (Philippart and Vranken 1983).

Barbel and dace, two rheophilic and relatively oxyphil cyprinids, demonstrate preferences for intermediate stream width values. When considering smaller to intermediate rivers, our results tend to demonstrate that, under otherwise comparable conditions, an increase in stream width for a given stream slope will tend to favor a particular fish community dominated by rheophilic cyprinids as a result of local increase in habitat diversity.

All seven species for which UDA shows high independent explanatory power exhibit a positive response to UDA. Unlike SLO, no clear replacement of species can be observed, even if species inhabiting slow-flowing waters appear to be more restricted to the downstream part of rivers (bleak and perch). This observation is in agreement with the view of a continual increase in the local richness towards downstream areas (Horwitz 1978; Rahel and Hubert 1991). This downstream increase in species diversity is usually related to environmental gradients having smooth transitions of abiotic factors contributing to nested patterns of assemblage composition along the longitudinal gradient (Sheldon 1968).

MAT is of first importance for six species and our optimal thermal ranges are in good agreement with previous literature (Alabaster and Llyod 1982; Philippart and Vranken 1983; Mann 1996). The spawning temperature range of brown trout (1–8 °C) fits well with their thermal response curve, while bullhead demonstrate an intermediate optimal thermal range (spawning temperature range 5–12 °C). Bleak, which spawn at higher temperatures (17–28 °C), are limited to the warmest river reaches. The thermal response of roach is similar, but with a wider thermal range (spawning thermal range 7–19 °C), whereas gudgeon and chub showed an almost linear increase in their occurrence with temperature. MAR never appeared as an important variable. This result, associated with the absence of a significant response to MAT for several species known to spawn early in the year (barbel, dace, and stone loach), appears to indicate the need for a more precise description of the thermal conditions and their variations throughout the year.

In conclusion, our results clearly demonstrate the capacity of this multiscale modelling approach to produce general habitat models and single predictions for an area as large and diversified as our four basins. We demonstrate that sensitivity to local and regional scale processes is species specific. Thus, the use of synthetic descriptors such as species richness may not be sufficient to evaluate the interactive effect of local and regional factors on fish community (Angermeier and Winston 1998).

For the moment, there is a gap between two kinds of literature on riverine fish communities: modelling studies mainly concerned with species–habitat relationships at the local scale and papers indicating that regional and local diversities are strongly linked in stream communities. There is a need to develop species-specific modelling approaches, integrating regional and local instream processes. Such studies are needed urgently because effective conservation and rehabilitation of stream biodiversity require proper understanding of the determinants of species distribution across multiple spatial scales.

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Epilithic algal abundance in relation to anthropogenic changes in phosphorus bioavailability and limitation in mountain rivers

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Abstract: Low-level cultural eutrophication (0.1–3.8 $\mu g \cdot L^{-1}$ increase in total phosphorus (TP)) of oligotrophic mountain rivers resulted in 4- to 30-fold increases in benthic algal abundance. Because anthropogenic P was more bioavailable than naturally occurring P, there were higher algal abundances downstream relative to upstream of nutrient point sources at a given P concentration. Neither TP nor soluble reactive P concentrations were indicative of P bioavailability. Of the measures studied, epilithic alkaline phosphatase activity was most strongly correlated with algal abundance, most indicative of P bioavailability and thus the most precise indicator of P limitation. Although changes in dissolved inorganic nitrogen (DIN) to P ratios in river water and carbon (C) to P ratios in epilithon were consistent with changes in algal abundance and nutrient limitation, published water DIN to TP and tissue C to P ratio thresholds did not always yield accurate predictions of the type or degree of nutrient limitation. Epilithic N to P ratios and algal growth on nutrient-diffusing substrates were also inexact measures of epilithic nutrient limitation but, unlike other measures, were not strongly correlated with algal abundance. Thus, the predictability of the benthic algal response to anthropogenic nutrient additions in oligotrophic rivers will be improved by using measures indicative of both nutrient limitation and bioavailability.

Résumé : Une eutrophisation culturelle de faible intensité (augmentation de 0,1–3,8 $\mu g \cdot L^{-1}$ du phosphore total, TP) de rivières oligotrophes de montagne entraine des accroissements par un facteur de 4–30 de l'abondance des algues benthiques. Parce que le P d'origine anthropique est plus biodisponible que le P naturel, il y a de plus fortes densités d'algues en aval qu'en amont des points d'origine des nutriments, pour une même concentration de P. Ni les concentrations de TP, ni celles du P réactif soluble ne reflètent la biodisponibilité de P. Des variables étudiées, c'est l'activité de la phosphatase alcaline dans l'épilithon qui possède la plus forte corrélation avec la biomasse des algues, qui reflète le mieux la biodisponibilité de P et qui est ainsi le meilleur indicateur de la limitation en P. Bien que les changements du rapport de l'azote inorganique dissous (DIN) sur P dans l'eau de rivière et du rapport carbone (C) sur P dans l'épilithon concordent avec les variations de l'abondance des algues et la limitation en nutriments, les valeurs publiées des seuils de DIN:TP dans l'eau et de C:P dans les tissus ne permettent pas toujours de prédire avec exactitude le type ou l'importance de la limitation en nutriments. Le rapport N:P dans l'épilithon et la croissance des algues sur des substrats à diffusion de nutriments sont aussi des mesures imprécises de la limitation en nutriments dans l'épilithon, mais, contrairement à d'autres variables, ils ne sont pas fortement corrélés à l'abondance des algues. Ainsi, l'utilisation de variables qui reflètent à la fois la limitation en nutriments et la biodisponibilité augmentera la possibilité de prédire la réaction des algues benthiques à l'addition de nutriments d'origine anthropique dans les rivières oligotrophes.

[Traduit par la Rédaction]

Introduction

Cultural eutrophication, resulting from excess inputs of nitrogen (N) and phosphorus (P) to aquatic systems, commonly leads to algal abundance that exceeds that desired for aesthetic reasons or for maintenance of ecological integrity. The ability to predict, explain, and mitigate changes in periphyton abundance in rivers is inexact relative to capabilities for phytoplankton in lake ecosystems. Nutrient supply may be more closely linked to phytoplankton abundances than to periphyton abundances because of diffusion limitations associated with benthic communities in lakes (e.g., Turner et al. 1994) and physical stressors (e.g., spates) in rivers (e.g., Biggs et al. 1998). However, in the absence of overriding physical or biological controls, N or P or both generally limit the abundance of benthic algae in rivers (Borchardt

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Table 1. Main advantages and potential considerations when using ratios of nitrogen to phosphorus concentrations (N:P) in water and algal tissue, alkaline phosphatase activity (APA), or nutrient-diffusing substrates (NDS) to determine nutrient-limiting benthic algal abundance.

Measure	Main advantages	Considerations
Water N:P	Predictable hyperbolic response of algal growth potential to changes in N:P (e.g., Schanz and Juon 1983)	Not relevant when nutrient-replete, may not reflect cellular nutrient ratios, small changes undetectable (Borchardt 1996); does not reflect tight periphyton–substrate nutrient cycling (Pringle 1987); can be only weakly predictive (Francoeur et al. 1999)
Tissue N:P	Robust community optimum (Redfield 1958); reflects food quality for higher trophic levels (e.g., Sterner and Hessen 1994; Stelzer and Lamberti 2002)	Optimum is species-specific (Rhee and Gothman 1980); grazing can change ratios (Hunter 1980); growth-rate-dependent (McMahon et al. 1974); can be only weakly predictive (Francoeur et al. 1999)
APA	Inverse function exists between APA and PO_4, total P (TP) and cellular TP (see Jansson et al. 1988)	Generally measures maximum potential APA production, difficult to completely separate from APA produced by bacteria and lysed cells, variable among species (but see Rengefors et al. 2003), also occurs in response to starvation for pyrimidines or for guanine, diurnal cycles can occur (Jansson et al. 1998)
NDS	Used successfully to address diverse theoretical and applied questions in benthology (Scrimgeour and Chambers 1997); early pulse mimics allochthonous decay (Pringle and Bowers 1984; Pringle 1987)	Diffusion low and inconsistent, pores may clog, sorb P, contain P-binding agents (Pringle 1987); difficult to partition species according to nutrient-supply ratios (Borchardt 1996); a qualitative not a quantitative assessment

1996). Yet despite strong evidence for nutrient control of benthic algal growth in many rivers, it is not clear how much variation can be explained by nutrient availability, and which nutrient(s) will limit growth in a given location (Dodds et al. 2002).

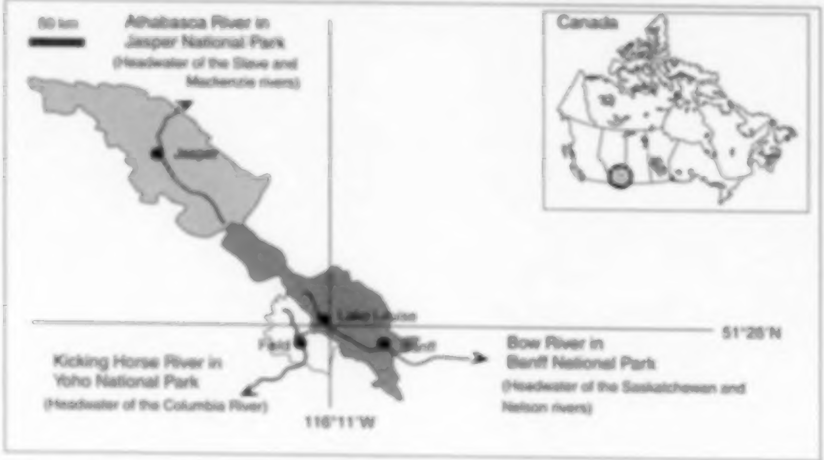
Indicators of nutrient limitation may poorly predict the response of periphyton to changes in nutrient availability in rivers because concentrations and forms of N and P in rivers are highly variable in space and time, are highly correlated, and can colimit algal growth (Francoeur 2001). Methods used to identify and quantify the nutrient status of algae include measurements of nutrient concentrations in stream water or algal tissue, physiological indicators or growth rates, and artificial enrichment of water or substrate. Each method has advantages and potential drawbacks (Table 1). For example, N to P ratios (N:P) in water or algae are often compared with the theoretical ideal molar ratio of 16:1 (e.g., Redfield 1958), but precise ratios have been shown to be species-specific (e.g., Kahlert 1998). Alkaline phosphatase activity (APA) measures the ability of organisms (including bacteria and algae) to cleave organically bound P and is usually indicative of the degree of P limitation, but being starved of certain proteins can elicit an APA response (e.g., Jansson et al. 1988). Relative algal growth on nutrient-diffusing substrates (NDS) filled with agar amended with nutrients and incubated in situ is often used to determine the type (but not the degree) of nutrient limitation in a given location (e.g., Pringle 1987).

In addition to potential problems in interpreting results from the use of a single indicator, the types or degree of limitation may vary because of differences in the scale of limitation or nutrient sources. For example, APA reflects cellular nutrient limitation, whereas NDS experiments infer community limitation. The P concentration found to saturate thin biofilms was two orders of magnitude lower than the concentration required to saturate benthic diatom mats (Bothwell 1988, 1989). Also, nutrient-limitation indicators may reflect nutrient availability in overlying water (water N:P), substrate (NDS), or both (APA, tissue N:P). Periphyton can respond to variations in nutrient supply in the water column, in the substrate alone, or in both (Pringle 1987). A comparison of methods is needed to identify practical limitations and choose appropriate methods for various situations and study objectives.

In P-limited systems, the relationship between P and algal abundance is particularly weak because there is a strong selective advantage for organisms that can use organically bound P, and tight P spiraling. For example, assessment of algal nutrient status from concentrations of total P (TP) and total N in water can overestimate nutrient availability because measures include organically bound fractions that cannot be used by biota. Conversely, the use of soluble-nutrient forms (soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN)) can underestimate bioavailability because certain organic nutrients can be utilized. Furthermore, available limiting nutrient(s) are quickly assimilated by organisms in oligotrophic systems, and therefore may not be detected in measures of river nutrient concentrations. Paul et al. (1991) concluded that because epilithon in rivers with the least bioavailable P reassimilated the P metabolites most quickly, empirical P-abundance relationships would underestimate algal development in the most oligotrophic rivers.

In this study, we assessed the efficacy of various indicators of nutrient limitation in explaining patterns of epilithic abundance in oligotrophic mid-sized (4th–5th order at 1 : 250 000 scale) mountain rivers upstream and downstream of four anthropogenic nutrient point sources. The amount of nutrient loading relative to river discharge varied among rivers and point sources and therefore provided both natural and anthropogenic gradients of nutrient loading to test nutrient-limitation measures. The goals of this study were to (*i*) compare the types and magnitudes of epilithon nutrient limita-

Fig. 1. Map of the Athabasca River within Jasper National Park boundaries, the Bow River within Banff National Park boundaries, and the Kicking Horse River within Yoho National Park boundaries, and the locations of Jasper, Lake Louise, Banff, and Field townsites in Alberta and British Columbia, Canada (51°10'N–53°54'N, 115°35'W–118°05'W; 1000–1500 m above sea level).



tion inferred from C:P and N:P in water and in epilithon tissue, APA, and NDS, (*ii*) determine whether the type or degree of limitation changes as a result of low-level (parts per billion) eutrophication, and (*iii*) determine whether nutrient-limitation measures are related to the gradient in anthropogenic nutrient additions and epilithic abundance.

Methods

Study sites

Methods to determine the type of nutrient (N or P) potentially limiting epilithon growth were used at sites upstream and downstream of discharges from municipal wastewater treatment plants (MWWTP) to the Athabasca, Bow, and Kicking Horse rivers in the National Parks in the Rocky Mountains of Alberta and British Columbia (Fig. 1). Detailed descriptions of the rivers, MWWTPs, subalpine and montane ecoregions, and sampling protocols are given in Bowman (2004). Briefly, the Athabasca River receives wastewater from the Town of Jasper, the Bow River receives wastewater from the towns of Lake Louise and Banff, and the Kicking Horse River receives wastewater directly from the Town of Field and indirectly from the Emerald Lake resort, which discharges wastewater into the Emerald River tributary. Sampling sites were located approximately 2 km upstream and 3 km downstream of each MWWTP effluent discharge. In the case of the Kicking Horse River, downstream samples were collected below the confluence of the Emerald River and reflect impacts from both the Field and Emerald Lake MWWTPs, except for APA, which was sampled above the confluence. All sampling sites contained at least three shallow (30 cm), fast-flowing (0.5 $m \cdot s^{-1}$) riffles within a 500-m reach, and substrate dominated by cobble (10–20 cm diameter). Samples were collected in late September (in the Bow River) and early October (in the Athabasca and Kicking Horse rivers) in 2000, when reduced light from riparian shading or high turbidity did not limit algal growth. Nutrient-chemistry measurements were collected in 1998–2000, NDS experiments were performed in the Athabasca and Bow rivers in October of 1998 and 1999, and all other samples were collected in 2000.

Epilithic communities were dominated by diatoms both upstream and downstream of MWWTP discharges, whereas the proportional abundance of the dominant primary consumer, mayflies, declined, owing to an increase in chironomids downstream of effluent discharges (Bowman 2004). Taxonomic analysis of benthic algal samples showed that assemblages were composed mainly of diatoms at all sites (both upstream and downstream of discharges) and on all sampling dates, but cyanophytes and chlorophytes were also present at most sites. Epilithic communities on NDS were similar to natural communities. At sites upstream of MWWTPs, the most common scrapers were mayflies (Heptageniidae, Ephemerellidae, and Baetidae), but there were also numerous caddisfly (Brachycentridae) and stonefly (Taeniopterygidae) scrapers. The abundance of mayflies, caddisflies, and stoneflies declined downstream of the Jasper MWWTP, and their proportional abundance declined downstream of all MWWTPs.

Water chemistry

Water samples were collected monthly from August to October and analyzed for TP, SRP, nitrate+nitrite-N, and ammonia-N following modified standard methods (University of Alberta Limnology Laboratory, Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9). Concentrations of TP (unfiltered) and SRP (filtered; Murphy and Riley 1962) were determined colorimetrically with a Varioan Cary 50 probe and a Milton Roy Model 1001 Plus spectrophotometer. Ammonia-N (unfiltered) and nitrate+nitrite-N (filtered) were determined colorimetrically with a TechniconTM AutoanalyzerTM II using automated Berthelot and cadmium reaction methods, respectively. Concentrations of nitrate+nitrite-N and ammonia-N were summed to give DIN. Estimated water-chemistry concentrations for sites downstream of MWWTPs were derived by multiplying mean monthly (August–October) concentrations in effluent by the effluent to river discharge ratio and adding upstream river concentrations. Estimated downstream nutrient concentrations are more indicative of nutrient availability than measured concentrations because historical daily records show that concentrations at upstream sites and in MWWTP effluents are consistent over time but concentrations at downstream sites are highly variable (Bowman 2004). Measured and expected molar N:P ratios in river water were compared with previously published nutrient-limitation thresholds. Summaries of the results of numerous studies on benthic algal nutrient enrichment show that the transition to P limitation occurs over a broad N:P range, from >10 to 30, and N limitation generally occurs at N:P < 10 (Allan 1995; Borchardt 1996).

Algal abundance

To collect epiphyton for chlorophyll *a* analyses, a template was used to demarcate a 9.6-cm^2 area of maximum epiphyton abundance (generally the downstream end of the cobble), and epiphyton was removed with a scalpel; this procedure was repeated on the same cobble to obtain a sample for determining algal biovolume. Epilithon collected from each of three cobbles was combined into a composite sample (one composite for chlorophyll *a* and another for algal-biovolume analyses). Three composite samples for chlorophyll *a* and one composite sample for algal biovolume were collected from each site. Samples for determining algal biovolume were immediately preserved in 5% formalin. Methods used to estimate algal biovolume are described in detail by Findlay and Kling (2004). Samples for measuring chlorophyll *a* were immediately frozen on dry ice and stored in the dark at –20 °C until analyzed. Samples for measuring chlorophyll *a* were blended with double-distilled water for 10 s, filtered onto GF/F filters, and extracted in boiling 90% ethanol for 7 min. Chlorophyll *a* concentrations were measured with a fluorometer (Model 10, Turner Designs, Sunnyvale, California).

Elemental composition

Composite 20-mL samples of epilithon were removed with a scalpel from three randomly chosen rocks in a riffle, at three riffles per site (for a total of three samples per site). Bryophytes were abundant only at the site downstream of Lake Louise, interfered with APA analysis, and were therefore excluded from samples. Epilithon samples for tissue nutrient content were freeze-dried at –60 °C (Edwards Super Modulyo Freeze Drier, Crawley, West Sussex, UK), ground to a uniform powder with a mortar and pestle, dried at 90 °C to a constant weight, and stored in a desiccator. Between 0.1 and 0.2 g (±0.0001 g) of epilithon sample was weighed for analysis of percent P and 1–4 µg (±0.0001 µg) of epilithon sample was used for analyses of percent N and C. P was extracted using persulfate oxidation followed by analysis using the acid molybdate technique (American Public Health Association 1992). The amount of C and N in samples was measured using a Model 440 elemental analyzer (Control Equipment Corporation, Cazenovia, New York). Benzoic acid and SRM NIST 1575 apple leaves (US National Institute of Standards and Technology) were used as quality-control standards for elemental analyses. All C:N:P ratios are reported on a molar basis. We compared the nutrient content of epilithon to P-limitation thresholds of C:P > 369 and N:P > 32 derived by Kahlert (1998).

Alkaline phosphatase activity

A composite sample of epilithon taken from a 9.6-cm^2 area on each of three cobbles was used to measure APA and chlorophyll *a* concentration; as with samples for epilithic nutrient content, three samples per site were collected and bryophytes were excluded. APA was measured following the phytoplankton method of Waiser and Bothwell (National Water Research Centre, 11 Innovation Boulevard, Saskatoon, SK S7N 3H5, unpublished data) modified for use with epilithon samples, using information in the literature (e.g., Healey and Hendzel 1979; Jansson et al. 1988). The epilithon sample was suspended in sterile filtered river water, refrigerated, and analyzed within 6 h. Samples were slowly agitated with a syringe to break up clumps, and filtered on GF/C filters (to collect bacteria; M.F. Bowman, unpublished data). Next, one half of the sample was resuspended in sterile filtered river water and heated to 35 °C in a water bath. Epilithon samples and blanks of filtered river water were saturated with 10 µmol·L^{-}1 4-methylumbelliferyl phosphate. Fluorescence occurs when phosphate is enzymatically cleaved from 4-methylumbelliferyl phosphate. Fluorescence was measured at increasing time intervals with a fluorometer (Model 10-AU-005, Turner Designs) until APA production stabilized over time. The fluorometer was set up with stacked emission filters (top: dominant λ 479.8; middle: dominant λ 569.5; bottom: 10% neutral density) and the ultraviolet lamp was set up with an excitation filter and a reference filter. The amount of chlorophyll *a* on the remaining half of the composite epiphyton sample was used to standardize enzyme activity. Based on comparisons between P-sufficient and P-deficient phytoplankton assemblages, Healey and Hendzel (1979) concluded that APA (nmol·µg chlorophyll a^{-1}·h^{-1}) >5 was indicative of severe P deficiency, 3–5 was indicative of slight deficiency, and <3 was indicative of P-replete conditions.

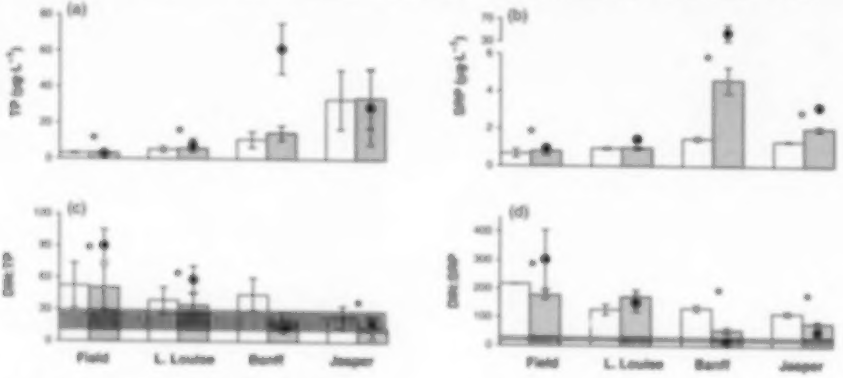
Nutrient-diffusing substrates

Clay pots were filled with agar made with P (0.5 mol·L^{-1} KH_2PO_4), N (0.5 mol·L^{-1} $NaNO_3$, in 1998 only), P plus N, or unamended solutions and sealed (for detailed methods see Scrimgeour and Chambers 1997). Amounts of P and N were sufficient to maintain nutrient diffusion for a 30-day period. Ten replicates of each NDS treatment were incubated in situ for the month of October (except for only 15 days in 1998 near Jasper, owing to a rapidly falling hydrograph) at sites both upstream and downstream of MWWTPs. Epilithon samples were removed for chlorophyll *a* analyses and a small subset of samples were analyzed for C, N, and P contents and APA. Hourly water temperature was recorded during each experiment using a thermister (Onset, Pocasset, Massachusetts), light attenuation was measured at the start and end of each experiment with a light meter (LI-COR, Lincoln, Nebraska), and velocity at each NDS was recorded with a current meter (Pygmy, JBS Energy, Inc, West Sacramento, California).

Statistical analyses

Data were log-transformed prior to analyses to correct for positive correlation of variance and mean if necessary. Dif-

Fig. 2. Total phosphorus (TP) (*a*), soluble reactive phosphorus (SRP) (*b*), ratio of dissolved inorganic nitrogen (DIN) to TP (*c*), and DIN:SRP ratio (*d*) (mean ± 1 standard error) measured upstream (open bars) and downstream (●) and estimated downstream (shaded bars) of wastewater discharges from Field, Lake Louise, Banff, and Jasper. Measured water-chemistry concentrations were calculated from monthly samples taken in August–October in 1998–2001. Estimated water-chemistry concentrations for sites downstream of wastewater discharges were derived by multiplying mean monthly (August–October) concentrations in effluent by the effluent to river discharge ratio, and adding upstream river concentrations. An asterisk indicates a significant difference ($p < 0.05$) between measured upstream and estimated downstream nutrient concentrations in a one-tailed paired (by month) *t* test. Hatched zones denote the N:P range where a switch from nutrient saturation to P limitation of periphyton occurs (Allan 1995; Borchardt 1996).



ferences in waster chemistry (TP, SRP, DIN:TP, DIN:SRP) were evaluated with randomized complete block analysis of variance (ANOVA) using MWWTP (Field, Lake Louise, Banff, Jasper), location (upstream or downstream), and month (August, September, October) as factors. Because there were significant interactions among factors, paired (by month) one-tailed *t* tests were used to evaluate upstream–downstream differences for a given MWWTP. Similarly, two-factor (MWWTP, upstream–downstream) ANOVAs were used to evaluate the significance of differences between chlorophyll *a* concentration and epilithic C:P and N:P and APA. Because significant interactions among factors occurred in each instance, two-sample one-tailed *t* tests were used to evaluate upstream–downstream differences in algal abundance, nutrient content, and APA. Analyses of covariance (ANCOVA) were used to determine whether P – algal abundance and P–APA relationships were different upstream and downstream of MWWTPs.

For NDS experiments run in 1998 and 1999, a repeated-measures ANOVA was used to evaluate differences in chlorophyll *a* accrual among NDS treatments. Because there were significant interactions among treatment, location (upstream or downstream), MWWTP, and year in the repeated-measures ANOVA, a one-way ANOVA followed by a Tukey's test was performed for each site. A one-way ANOVA was also used to evaluate the results of the NDS experiments conducted upstream and downstream of Field in 2000. Correlations among various measures of algal abundance, nutrient concentrations in river water, and epilithic nutrient limitation were calculated.

Results

At upstream sites, estimates of average TP concentrations over the period of algal accrual (August–October) ranged from 3.1 to 34 µg·L^{-1} and were lowest near Field and progressively higher near Lake Louise, Banff, and Jasper (Fig. 2*a*). Much of the TP at sites near Jasper is associated with periods of high turbidity in summer and would not be biologically available (e.g., bound with glacial flour). Nutrient concentrations, both measured and estimated, were generally slightly elevated with respect to upstream sites (TP, SRP, and DIN were elevated by 0.00–3.75, 0.03–3.14, and 0.7–23.5 µg·L^{-1}, respectively), with the exception of concentrations measured downstream of Banff, which were greatly elevated because of MWWTP malfunctions during sampling (Figs. 2*a*, 2*b*). On average, the contribution of ammonia-N to DIN was 92.3%, 33.0%, 24.4%, and 51.8% upstream and 80.1%, 30.6%, 43.0%, and 58.3% downstream of Field, Lake Louise, Banff, and Jasper, respectively.

Comparisons of mean DIN:TP and DIN:SRP molar ratios in river water, and reported P-limitation thresholds (N:P > 10–30; Allan 1995; Borchardt 1996), indicated that epilithon at all upstream sites (with one exception upstream of Jasper) would be limited by unavailability of P (Figs. 2*c*, 2*d*; Table 2). Both N:P ratios also indicated that epilithon at sites downstream of Field and Lake Louise would likely remain P-limited. Downstream of Banff and Jasper, average DIN:TP indicated that epilithon would not be P-limited, whereas mean DIN:SRP indicated that epilithon would be P-limited.

As expected, there were significant ($p < 0.05$) increases in chlorophyll *a* concentration downstream of all MWWTP effluent discharges (Fig. 3*a*). With the exception of the site downstream of Field, there were also increases in algal biovolume at all downstream sites (Fig. 3*a*). However, at a given estimated TP or SRP concentration, downstream sites always had higher chlorophyll *a* concentrations (or algal biovolume) than upstream sites (Figs. 3*b*–3*e*). ANCOVA showed that the slopes of chlorophyll *a* – TP and algal biovolume – TP relationships were significantly different ($p = 0.04$ and $p = 0.001$, respectively) upstream and downstream of MWWTPs. It was inappropriate to use ANCOVA for algal abundance – SRP relationships because of nonlinearity.

Table 2. The type of epilithic nutrient limitation (phosphorus (P), nitrogen (N), colimitation, or nutrient saturation) concluded from experiments with molar ratios of dissolved inorganic nitrogen (DIN) to total phosphorus (TP) or soluble reactive phosphorus (SRP) in water, molar C:P and N:P ratios in epilithic tissue, epilithic alkaline phosphatase activity (APA), and nutrient-diffusing substrates (NDS) upstream and downstream of municipal wastewater treatment plant discharges.

	P-limitation threshold					
	>10–30	>10–30	C:P > 369	N:P > 32	>3 (slight); >5 (severe)	
	DIN:TP[a]	DIN:SRP[a]	Tissue C:P[b]	Tissue N:P[b]	APA[c]	NDS[d]
Field						
Upstream	P	P	P	P	Severe P	**Saturation**
Downstream	P	P	P	P	Severe P	P
Lake Louise						
Upstream	P	P	P	P	Severe P	P
Downstream	P	P	P	P	Slight P	**Colimitation**
Banff						
Upstream	P	P	P	P	Severe P	**Colimitation**
Downstream	Saturation	**P**	**P**	Saturation	P saturation	Saturation
Jasper						
Upstream	**Saturation**	P	P	**Saturation**	Severe P	P
Downstream	Saturation	**P**	Saturation	**P**	P saturation	Saturation

Note: Discrepancies are indicated in boldface type.

[a] Allan (1995); Borchardt (1996).

[b] Kahlert (1998).

[c] Healey and Hendzel (1979).

[d] Significant in Tukey's test.

Internal measures of nutrient limitation (tissue C:N:P, APA) also suggested that epilithon was more limited by P availability at sites upstream of MWWTPs than at downstream sites (Table 2). The C:P and N:P contents of epilithon were significantly higher at sites upstream than downstream of MWWTPs (except near Banff and Jasper) (Figs. 4*a*, 4*b*). In the case of Jasper, nutrient ratios in epilithon collected from cobble and NDS were consistent; C:P and N:P were four- to six-fold lower for epilithon collected from substrates emitting P and N+P than for epilithon collected from control NDS, and were lower downstream than upstream of MWWTPs in a given NDS treatment (Figs. 4*c*, 4*d*). Comparison of tissue C:P and N:P with reported P-limitation thresholds for periphyton (C:P > 369, N:P > 32; Kahlert 1998) suggested that all upstream sites except the site near Jasper (based on N:P) would be P-limited (Figs. 4*a*, 4*b*, Table 2). Epilithic nutrient content also suggested that sites downstream of Field and Lake Louise would remain P-limited. However, contrary to other measures, tissue C:P downstream of Banff was just above the P-limitation threshold, and tissue N:P was below the P-limitation threshold upstream but above the threshold downstream.

Average APA of epilithon at upstream sites was significantly higher than at sites downstream of the Lake Louise, Banff, and Jasper MWWTPs (Fig. 5*a*). APA of epilithon from all upstream sites and the site downstream of Field was >5 nmol·μg chlorophyll a^{-1}·h^{-1}, indicative of severe P limitation (Table 2; Healey and Hendzel 1979). APA downstream of Lake Louise was in the 3–5 nmol·μg chlorophyll a^{-1}·h^{-1} range indicative of slight P limitation, while APA at sites downstream of Banff and Jasper was <3 nmol·μg chlorophyll a^{-1}·h^{-1}, indicative of P-replete conditions. As expected, epilithon grown on NDS incubated upstream of Field, emitting P or N+P, had APA in the range considered P-replete (Fig. 5*b*). There were not enough algae on control NDS to measure APA. Consistent with trends in algal abundance, APA at a given estimated TP or SRP concentration was higher upstream than downstream of MWWTPs (Figs. 5*c*, 5*d*; $p = 0.03$ for differences between slopes in ANCOVA for both APA–TP and APA–SRP relationships).

Overall, average epilithic accrual on NDS was greatest on N+P treatments in 11 of 16 experiments (Fig. 6). NDS experiments showed that algal growth was consistently N- and P-saturated at sites downstream of Jasper and Banff (Figs. 6*e*–6*h*; Table 2). Upstream of Jasper and Banff, unavailability of P or N+P limited algal abundance in 1999; the same trend, although not statistically significant, was also observed in 1998. Algal accrual upstream and downstream of Lake Louise was also constrained by insufficient P or N+P but this trend was not significant upstream in 1999, when there was very little algal growth on NDS (Figs. 6*c*, 6*d*). The results of the NDS experiment near Field were inconclusive, owing to high variability within treatments and very low algal growth at upstream sites, both of which limited statistical resolution (Fig. 6*a*). P limited algal abundance downstream of Field and the Emerald River confluence (Fig. 6*b*); the same trend, although not statistically significant, was also observed downstream of Field but above the Emerald River confluence (Fig. 6*a*). During incubations of NDS, water temperatures were 3–8 °C in 1998, 3–5 °C in 1999, and 3–8 °C in 2000. There were no significant differences in light attenuation, temperature, or velocity between sites upstream and downstream of MWWTPs, and average velocity did not vary among NDS treatments within a site ($p > 0.05$; M.F. Bowman, unpublished data).

Measures of algal abundance (chlorophyll *a* concentration and biovolume) were highly correlated ($r > 0.7$) with estimated DIN:SRP and DIN:TP, C:P and C:N of epilithon, and

Fig. 3. Epilithic chlorophyll *a* concentration (mean ± 1 standard error) and algal biovolume (●) at sites upstream (open bars) and downstream (shaded bars) of wastewater discharges in Field, Lake Louise, Banff, and Jasper in autumn 2000 (an asterisk indicates a significant difference ($p < 0.05$) between upstream and downstream sites in a one-tailed *t* test) (*a*), and chlorophyll *a* concentration versus estimated TP (*b*) and SRP (*c*) and algal biovolume versus estimated TP (*d*) and SRP (*e*) at upstream (open symbols) and downstream (shaded symbols) sites.

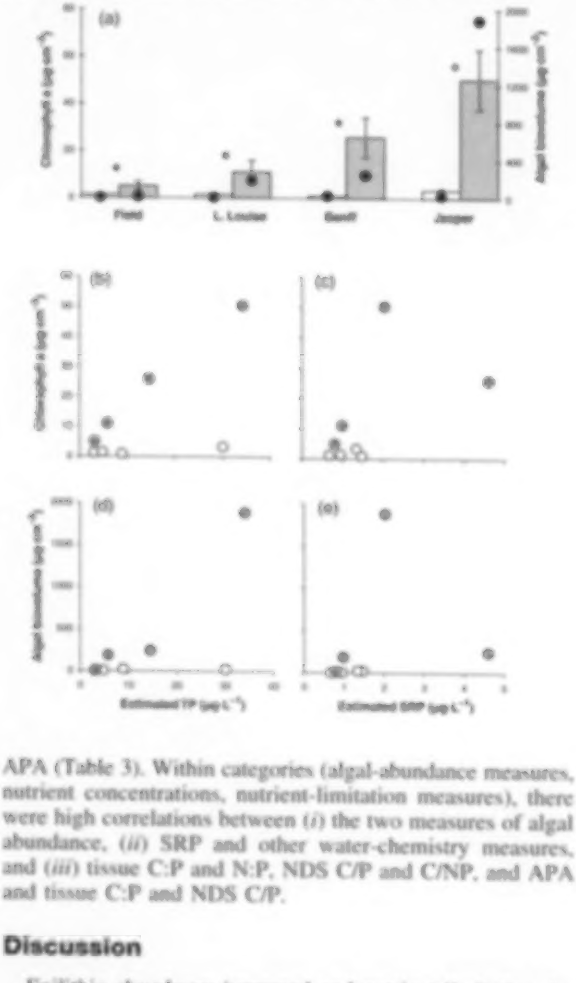
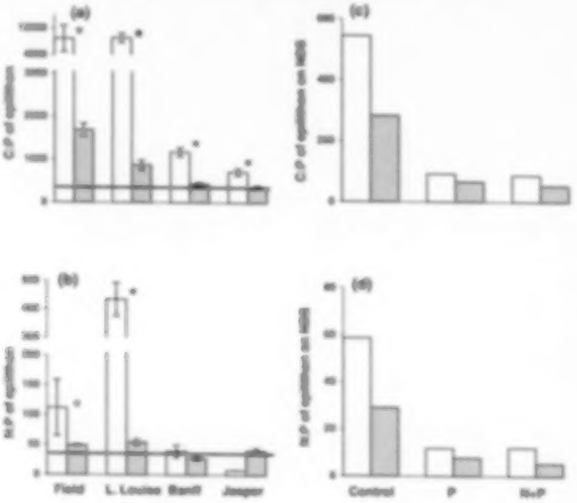


Fig. 4. Molar ratios of carbon to phosphorus (C:P) (*a*) and nitrogen to phosphorus (N:P) (*b*) (mean ± 1 standard error) in epilithon sampled upstream (open bars) and downstream (shaded bars) of wastewater discharges, on natural substrates in autumn 2000 (horizontal lines indicate the P-limitation thresholds of Kahlert (1998); an asterisk indicates a significant difference ($p < 0.05$) between upstream and downstream sites in a one-tailed *t* test). C:P (*c*) and N:P (*d*) molar ratios of epilithon grown on a subset of P and N+P nutrient-diffusing substrate (NDS) treatments incubated upstream (open bars) and downstream (shaded bars) of the Jasper wastewater discharge in autumn 1999.



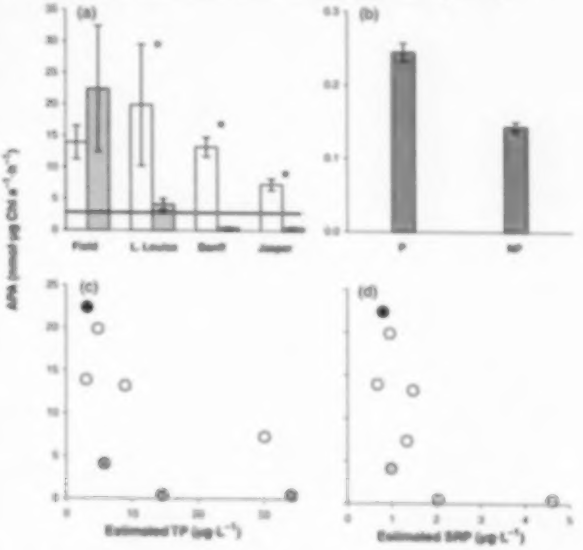
APA (Table 3). Within categories (algal-abundance measures, nutrient concentrations, nutrient-limitation measures), there were high correlations between (*i*) the two measures of algal abundance, (*ii*) SRP and other water-chemistry measures, and (*iii*) tissue C:P and N:P, NDS C/P and C/NP, and APA and tissue C:P and NDS C/P.

Discussion

Epilithic abundance increased and nutrient limitation decreased as a result of low-level anthropogenic eutrophication of nutrient-poor mountain rivers. Nutrient concentrations in river water and epilithic enzyme activity, nutrient content, and abundance on artificially enriched substrates showed that epilithon in the three oligotrophic mountain rivers was constrained by insufficient P at sites upstream of significant pollution sources. In contrast, sites downstream of MWWTPs showed a variable response, from severe P limitation to P saturation. The observation that epilithic abundance increased and nutrient limitation decreased as a result of low-level eutrophication was consistent with earlier findings (e.g., Bothwell 1989; Scrimgeour and Chambers 2000). However, contrary to expectations, at a given P concentration epilithic abundance was higher and the degree of nutrient limitation lower at sites downstream than at sites upstream of MWWTPs; this suggests that neither TP nor SRP concentrations precisely reflect P bioavailability.

Although nutrient concentrations in river water and their ratios were an imprecise reflection of nutrient bioavailability, the use of mean DIN:TP ratios resulted in accurate predictions (with one exception) of whether epilithon would be P-limited or P-saturated. Conversely, DIN:SRP ratios suggested that benthic algae in the most nutrient-rich sites (downstream of Banff and Jasper) were P-limited, when three other measures of nutrient limitation suggested nutrient saturation. Borchardt (1996) concluded that water nutrient ratios are indicative of nutrient limitation provided absolute nutrient concentrations are lower than growth-limiting levels. Although absolute concentrations of SRP estimated at all sites (mean = 1.6 µg·L^{-1}, standard error = 1.3 µg·L^{-1}) at all sites were considerably less than concentrations found by others to saturate benthic periphyton mats (>30–50 µg·L^{-1}, Bothwell 1989; >25–35 µg·L^{-1}, Chambers et al. 2000), estimated DIN concentrations at all sites (mean = 54–111 µg·L^{-1}) were in excess of those found to saturate periphyton in nearby river reaches (DIN > 50 µg·L^{-1}; Chambers et al. 2000). Because high N concentrations would cause elevated N:P at all sites, the ability to detect a shift between P limitation and saturation using N:P would not be precise in N-replete systems. However, consistent with previous findings

Fig. 5. Alkaline phosphatase activity (APA) of epilithon on natural substrates (mean ± 1 standard error) sampled upstream (open bars) and downstream (shaded bars) of wastewater discharges in autumn 2000 (the horizontal line is the P-limitation threshold derived by Healey and Hendzel (1979); an asterisk indicates a significant difference ($p < 0.05$) between upstream and downstream sites in a one-tailed *t* test) (*a*) and epilithon grown on P and N+P NDS treatments incubated upstream of Field (*b*). APA versus estimated TP (*c*) and SRP (*d*) at sites upstream (open symbols) and downstream (shaded symbols) of wastewater discharges and downstream of the Field wastewater discharge but upstream of the Emerald Lake wastewater discharge (solid symbol).



for lake ecosystems (Morris and Lewis 1988; Axler et al. 1994), we found that nutrient-limitation predictions based on DIN:TP generally agreed with other measures of nutrient limitation, and may be more indicative of bioavailable fractions of P than DIN:SRP in P-limited systems.

APA was the only measure of nutrient limitation studied that directly measured the propensity of benthic organisms to cleave organically bound P, and accurately discriminated among various degrees of P limitation. Although most knowledge of APA as a P-deficiency indicator comes from work on phytoplankton in lakes and oceans (e.g., Healey and Hendzel 1979), it has been shown experimentally that APA increases the P supply to algae in streams (Klotz 1985, 1991), and APA of epilithon is directly related to streamwater N:P (Klotz 1992). Consistent with a general review of APA as a P-deficiency indicator (Jansson et al. 1988), our results showed that APA was inversely related to TP and SRP concentrations and the cellular P level.

In contrast with algal nutrient status identified by APA and NDS, predictions based on epilithic C:P and N:P at the three more-P-rich sites (average molar DIN:SRP ratio in river water = 56–115) were not consistent with other measures. Francoeur et al. (1999) also found that the cellular nutrient content of periphyton was of limited use for predicting which nutrient limited NDS bioassays in relatively P-rich rivers (DIN:SRP = 15–126). However, results obtained at our most P-limited sites (DIN:SRP = 126–216) are consistent with the conclusion from experiments using river water with relatively low P concentration (DIN:SRP = 100–231) that the degree of P limitation based on periphytic nutrient content was consistent with other measures of nutrient limitation, including APA (Bothwell 1985). In contrast with epilithic N:P, epilithic C:P was highly correlated with algal abundance and epilithic APA. C:P ratios of benthic periphyton identified as P-replete were higher in the mountain rivers (C:P < 440) than those of benthic algae from other systems (C:P < 158; Kahlert 1998), but C:P ratios of epilithon identified as P-limited (C:P > 720) were not inconsistent with the C:P ratio of phytoplankton collected in a highly oligotrophic lake (250–1750; Elser and George 1993). If P concentrations are limiting, tissue C:P and N:P would be expected to increase because algae make more efficient use of P incorporated into cells (e.g., Hecky et al. 1993). Thus, algal communities adapted to lower nutrient concentrations will have sufficient nutrient supplies at higher C:P ratios than algal communities in more P-replete systems.

The amount of epilithon on control relative to treatment NDS was not always consistent with other measures of nutrient limitation, and was not highly correlated with algal

Fig. 6. Chlorophyll *a* concentrations (mean + 1 standard error) on control (C), phosphorus (P), nitrogen (N), and P+N (NP) NDS incubated in rivers upstream (open bars) and downstream (shaded bars) of wastewater discharges in 1998–2000 (bars with the same letters were statistically equivalent in a Tukey's test).

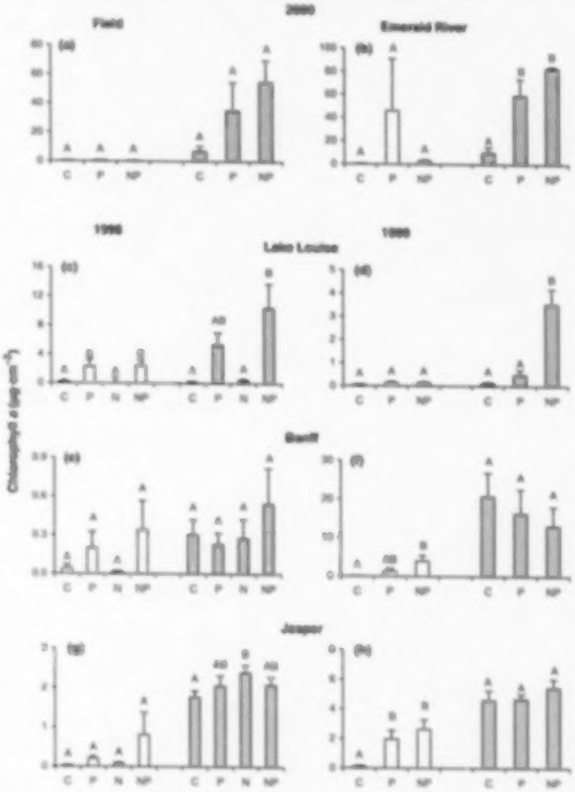


Table 3. Correlations between measures of algal abundance (chlorophyll *a* concentration, algal biovolume), measured and estimated water nutrient concentrations (TP, SRP, DIN), elemental composition of epilithon (C:P, C:N, N:P), amount of chlorophyll *a* on the control divided by treatment nutrient-diffusing substrates (C/P, C/NP), and alkaline phosphatase activity.

				Measured					Estimated					Tissue			NDS	
		Chlorophyll *a*	Algal biovolume	TP	SRP	DIN	DIN:SRP	DIN:TP	TP	SRP	DIN	DIN:SRP	DIN:TP	C:P	C:N	N:P	C/P	C/NP
		+	+	+	+	+	–	–	+	+	+	–	–	–	–	–	+	+
Algal biovolume	+	**0.92**																
Measured in river water																		
TP	+	0.66	**0.72**															
SRP	+	0.63	0.54	**0.77**														
DIN	+	0.49	0.36	0.52	**0.88**													
DIN:SRP	–	**–0.71**	–0.70	**–0.88**	**–0.92**	–0.64												
DIN:TP	–	–0.56	–0.59	**–0.93**	**–0.75**	–0.39	**0.91**											
Estimated for river water																		
TP	+	0.56	0.70	**0.88**	0.39	0.11	–0.62	**–0.81**										
SRP	+	0.65	0.65	**0.88**	**0.96**	**0.78**	**–0.96**	**–0.86**	0.6									
DIN	+	0.46	0.53	0.68	**0.83**	**0.84**	–0.70	–0.54	0.37	**0.84**								
DIN:SRP	–	**–0.75**	**–0.72**	**–0.88**	**–0.88**	–0.64	**0.96**	**0.89**	–0.68	**–0.95**	–0.63							
DIN:TP	–	**–0.82**	**–0.86**	**–0.88**	–0.56	–0.25	**0.79**	**0.85**	**–0.90**	–0.70	–0.38	**0.84**						
Tissue nutrient content																		
C:P	–	**–0.76**	**–0.85**	**–0.80**	–0.56	–0.53	0.60	0.55	**–0.76**	–0.68	–0.69	0.66	**0.75**					
C:N	–	**–0.71**	**–0.76**	–0.36	–0.45	–0.32	0.55	0.31	–0.28	–0.49	–0.29	0.60	0.54	0.50				
N:P	–	–0.33	–0.40	–0.64	–0.30	–0.37	0.26	0.37	–0.66	–0.40	–0.55	0.31	0.45	**0.78**	–0.14			
Nutrient-diffusing substrates																		
C/P	+	0.61	0.50	0.57	**0.74**	0.48	**–0.82**	**–0.73**	0.35	**0.72**	0.51	**–0.73**	–0.59	–0.27	–0.32	–0.01		
C/NP	+	0.49	0.32	0.37	0.61	0.41	–0.65	–0.57	0.18	0.55	0.36	–0.58	–0.42	–0.08	–0.16	0.10	**0.96**	
Alkaline phosphatase activity																		
APA	–	**–0.90**	**–0.91**	**–0.85**	**–0.76**	–0.51	**0.88**	**0.79**	–0.70	**–0.82**	–0.66	**0.84**	**0.88**	**0.77**	0.59	0.42	**–0.77**	–0.59

Note: Correlations greater than 0.70, shown in boldface type, are statistically significant ($p < 0.05$); to maintain an overall p value of 0.05, a Bonferroni correction requires a correlation of greater than 0.92 (underlined). The sign (+ or –) indicates an expected relationship with the eutrophication gradient; multiplying row and column signs gives the expected sign of the relationship between parameters.

abundance. Discrepancies occurred at sites downstream of Lake Louise and upstream of Banff, when NDS experiments suggested colimitation by P and N but all other nutrient-limitation measures indicated P limitation. These sites were midway along the gradient of river P concentrations, representing the transition between P limitation and P saturation. The fact that the site downstream of Lake Louise was slightly P-limited (based on APA) supports the idea that this site was closer to the P-limitation threshold than other sites. Therefore, like N:P (Borchardt 1996), NDS near nutrient-limitation thresholds may not be as precise as other measures of nutrient limitation. However, growth of epilithon on NDS did highlight the fact that the response of multispecies communities to enrichment with a single nutrient is less frequent and detectable than the response to enrichment by both N and P (Elser et al. 1990; Francoeur 2001).

In conclusion, changes in epilithic P limitation were most accurately identified using APA, and patterns in APA and river-water DIN:TP and epilithic C:P were consistent with patterns in algal abundance and nutrient limitation. The amount of P bioavailable to epilithic organisms is reflected in the APA, DIN:TP, and epilithic C:P measures of nutrient limitation. Given the low concentrations of bioavailable P that naturally occur in mountain rivers (<1.5 $\mu g \cdot L^{-1}$ SRP), the use of indicators that incorporate the full range of P available to primary producers is critical when assessing the nutrient status of these systems. This study also illustrated that APA thresholds developed to predict the nutrient status of phytoplankton are appropriate for benthic algae, and that APA is particularly useful because it identifies the degree of P limitation along eutrophication gradients. More accurate calibration of other nutrient-limitation measures for nutrient-poor systems would also be valuable (e.g., epilithic C:P for detection of P limitation in P-limited environments). Because algal abundance was correlated with the degree of P limitation, mitigation of anthropogenic P loading is expected to decrease algal abundance in the mountain rivers.

However, the observation that for a given nutrient concentration, benthic algae were more abundant, had lower APA, and exhibited less nutrient limitation at sites downstream than upstream of effluent discharges shows that P concentrations alone are not predictive of the abundance of benthic biota. Because the use of phosphatase enzymes is metabolically costly, organisms produce APA only when bioavailable P is scarce. Therefore, lower APA at downstream sites suggested that P was more bioavailable downstream than upstream of effluent discharges. When P is more available to organisms, the need to produce APA decreases, less P per unit growth is needed because organisms have higher growth efficiencies (lower metabolic costs), and the degree of P limitation declines. Thus, the greater amount of bioavailable P downstream of MWWTPs explains why higher algal abundances were observed downstream of effluent discharges even though nutrient concentrations were comparable to those at sites above MWWTPs. Previous work on phytoplankton in lakes and oceans has also clearly shown that "new" nutrients in a system can stimulate "new" productivity (e.g., Caraco et al. 1992). Therefore, an improved understanding of nutrient bioavailability and limitation will improve the ability to explain patterns in periphyton abundance along nutrient gradients in oligotrophic rivers.

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Chemical changes to leaf litter from trees grown under elevated CO_2 and the implications for microbial utilization in a stream ecosystem

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Abstract: Chemical alterations to leaf litter associated with growth under elevated CO_2 may impact aquatic ecosystems that rely on terrestrial leaf litter as a carbon source. This study examined how elevated CO_2 altered the chemistry and subsequent response of stream microorganisms growing on the leaf litter of three riparian tree species. Quaking aspen (*Populus tremuloides*), white willow (*Salix alba*), and sugar maple (*Acer saccharum*) were grown under ambient (360 parts per million) and elevated (720 parts per million) CO_2 for an entire growing season and senesced leaf litter was incubated in a stream for 80 days. Elevated-CO_2 effects on the chemistry of senesced litter were species-specific. Aspen leaves contained higher concentrations of lignin, maple leaves contained higher concentrations of soluble phenolic compounds, and willow leaves contained higher concentrations of carbohydrate-bound condensed tannins. Initially higher concentrations of soluble phenolic compounds in maple leaves were rapidly leached in stream water. However, higher concentrations of carbohydrate-bound tannins in elevated-CO_2-grown willow leaves persisted and were correlated with reduced phenol oxidase activities of attached microbiota. Overall, altered leaf chemistry associated with growth under elevated CO_2 did not strongly suppress microbial activity during stream incubation. In cases where there was evidence of suppression, it was largely species-specific.

Résumé : L'altération chimique de la litière de feuilles dans des conditions de croissance sous des concentrations élevées de CO_2 peut affecter les écosystèmes aquatiques qui dépendent de l'apport de litière de feuilles terrestres comme d'une de leurs sources de carbone. Notre étude examine comment un accroissement du CO_2 modifie les réactions chimiques et, par conséquent, les réactions des microorganismes d'eau courante qui se développent sur la litière de feuilles de trois espèces d'arbres riverains. Nous avons cultivé des peupliers faux-trembles (*Populus tremuloides*), des saules blancs (*Salix alba*) et des érables à sucre (*Acer saccharum*) dans des conditions de CO_2 ambiant (360 parties par million) et dans des conditions élevées (720 parties par million) durant une saison entière de croissance; nous avons ensuite incubé la litière parvenue à l'état sénescent dans un cours d'eau pendant 80 jours. Les effets de l'augmentation du CO_2 sur la chimie de la litière sénescente varient d'une espèce à l'autre. Les feuilles de tremble contiennent plus de lignine, celles d'érable des concentrations plus élevées de composés phénoliques solubles et celles de saule des teneurs plus grandes de tanins condensés liés aux hydrates de carbone. Les concentrations initiales plus fortes de composés phénoliques solubles dans les feuilles d'érable sont vite lessivées dans l'eau de cours d'eau. Cependant, les concentrations plus grandes de tanins liés aux hydrates de carbone dans les feuilles de saules cultivés dans des conditions élevées de CO_2 se maintiennent et sont en corrélation avec l'activité réduite de la phénol oxydase dans les microorganismes qui leur sont associés. En général, la modification de la chimie des feuilles dans les conditions de croissance sous des concentrations élevées de CO_2 ne diminue pas de façon importante l'activité microbienne durant l'incubation dans un cours d'eau. Dans les cas où il y a des indices d'une diminution, cette diminution varie en grande partie d'une espèce à l'autre.

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Introduction

Rising levels of atmospheric CO_2 will potentially have far-reaching impacts on both terrestrial and aquatic ecosystems. A doubling in atmospheric CO_2 by the end of this century (Houghton et al. 2001) may lead to increased rates of carbon fixation by vascular C_3 plants (e.g., Gill et al. 2002). This decrease in carbon limitation will likely be accompanied by a shift to limitation by other nutrients such as nitrogen in many ecosystems. Under these conditions plants may allocate more carbon to secondary compounds like tannins, while nitrogen-rich compounds become more dilute (e.g., Wetzel and Grace 1983; Lindroth et al. 1993; Strain and Bazaaz 1983).

In addition to potentially having negative effects on terrestrial herbivores (e.g., Lindroth et al. 1993; Lindroth and Kinney 1998) and the decomposition of leaf litter in terrestrial environments (e.g., Cotrufo and Ineson 1996; De Angelis et al. 2000), elevated-CO_2-induced alterations in the chemistry of deciduous leaves may also negatively impact many aquatic ecosystems. Headwater streams as well as larger rivers depend on subsidies of allochthonous organic matter for up to 99% of the annual carbon budget (Minshall 1967; Fisher and Likens 1973; Wetzel 2001). Therefore, alterations in the chemistry of terrestrial leaf litter that result in increased resistance to decomposition will potentially influence the chemical composition of and capacities for enzymatic hydrolysis and oxidation by microbiota in these ecosystems. This hypothesis has been supported in several short-term studies where quaking aspen (*Populus tremuloides* Michaux) was grown under both ambient (360 parts per million (ppm)) and elevated (720 ppm) concentrations of atmospheric CO_2, the naturally senesced leaf litter being subsequently incubated in a stream (Tuchman et al. 2002, 2003*b*; Rier et al. 2002) or a laboratory microcosm (Wetzel and Tuchman 2004). For example, Tuchman et al. (2002) demonstrated that elevated-CO_2-grown quaking aspen leaves placed in a stream for 14 days had less than half of the biomass-specific bacterial productivity of those incubated under ambient CO_2. Rier et al. (2002), also working with quaking aspen leaves, found negative effects on the microbial community in the first 12 days of incubation. However, between 12 and 30 days, it appeared that a combination of leaching of soluble phenols and colonization by aquatic fungi, which increased leaf nitrogen, might compensate for any initial chemical differences between elevated- and ambient-CO_2-grown leaves.

Although the above studies indicate that an atmosphere enriched with CO_2 could indirectly affect leaf-litter quality and subsequent microbial utilization, at least initially, a need exists to evaluate the ubiquity of such effects in stream ecosystems. Litter from several tree species needs to be assessed, the effects on stream microorganisms need to be determined over longer periods of time (over 30 days), and potential compensatory mechanisms such as those shown by Rier et al. (2002) need to be investigated further.

The objective of the current study was to examine how elevated CO_2 altered the chemistry and the subsequent response of stream microorganisms to the leaf litter of three tree species common to the riparian zones of northern Michigan streams. The three species, chosen on the basis of decomposition rates reported in the literature (sensu Petersen and Cummins 1974), were quaking aspen (slow decomposition rate), white willow (*Salix alba* L.) (moderate decomposition rate), and sugar maple (*Acer saccharum*) (fast decomposition rate). Initial chemical differences between the leaf litter of ambient- and elevated-CO_2-grown trees were assessed, and changes in bacterial biomass, fungal biomass, phenol oxidase activity (POA), and leaf chemistry were followed throughout an 80-day in-stream incubation.

Methods

Tree growth under elevated CO_2

Quaking aspen, white willow, and sugar maple were grown at The University of Michigan Biological Station in northern Lower Michigan. Although the white willow is not native to northern Michigan, it is found in the riparian zone of the stream where the in-situ part of this experiment was run. Sixteen 6-year-old quaking aspen clones, twenty 2-year-old white willow clones, and twenty 2-year-old sugar maple siblings (collected under one parent tree) were grown in open-bottom root boxes containing a soil homogenate of 80% native Kalkaska rubicon sand and 20% topsoil. This mixture provided nutrient levels that were comparable to those of soils in this region (Zak and Pregitzer 1990). Trees were enclosed in 1-m^3 open-top chambers, air being circulated through each with a blower fan (see Curtis and Teeri 1992). Half of the chambers were maintained at ambient atmospheric CO_2 concentrations (360 ppm), while half were maintained at elevated levels (720 ppm) by dispensing 100% CO_2 into the blower fans. Elevated CO_2 concentrations were maintained by continuously monitoring CO_2 concentrations in each elevated-level and one ambient-level chamber with an infrared gas analyzer (LICOR® model LI-6252, Lincoln, Nebraska, USA) and then periodically adjusting the flow of 100% CO_2 into each chamber. Treatments were maintained through the entire growing season, naturally senesced leaf litter being collected at the end of the season.

In-stream incubation

Leaf litter was incubated in a third-order section of the East Branch Maple River (45°34.505′N, 84°44.706′W) near Pellston, Michigan. The East Branch Maple River originates from Douglas Lake approximately 3 km northeast of the study site, where it flows through undeveloped wetlands and northern hardwood forests. Nitrogen and phosphorus concentrations were low in this section of stream, with a mean nitrate concentration of 13 μg $N \cdot L^{-1}$, a mean ammonia concentration of 25 μg $N \cdot L^{-1}$, and a mean soluble reactive phosphorus concentration of 2.2 μg $P \cdot L^{-1}$ (R. Vande Kopple, University of Michigan Biological Station Stream Research Facility, 9008 Biological Road, Pellston, MI 49769, USA, unpublished data). Mean stream width in the study reach was 5 m and mean depth was 0.5 m.

Incubation was conducted between February and April 2002. Ambient- and elevated-CO_2-grown leaves of each species were placed individually in 1.4-mm-mesh bags (n = 120 bags) to discourage macroinvertebrate colonization, and suspended in the East Branch Maple River at a current velocity of ~20 $cm \cdot s^{-1}$. Six ambient- and six elevated-CO_2-grown leaves of each species were retrieved on days 10, 20, 40, and 80. Stream temperature was monitored with an Onset Stow-Away® XTI temperature recorder (Onset Computer, Bourne, Massachusetts, USA) set to take a reading once every hour.

Leaves collected from the stream were placed in individual zipper bags filled with stream water and immediately transported to the laboratory. Leaves were removed from the mesh bags, gently rinsed in stream water to remove silt and fine particulate organic matter, and then subsampled with a 7.2 mm diameter cork borer to determine bacterial productivity (composite sample of five disks), bacterial biomass (composite sample of five disks), fungal biomass (composite sample of five disks), and POA (one 31 mm diameter leaf disk). The remaining leaves were assayed for total phenols (TPh), soluble condensed tannins (CT), carbohydrate-bound condensed tannins (BT), the ratio of organic carbon to organic nitrogen (C:N), and lignin.

Microbial assays

Bacterial productivity was determined by measuring the incorporation of ^{3}H-leucine into protein. Leaf disks were first briefly sonicated for <30 s in 10 mL of sterile filtered (0.2 µm pore size) stream water to remove bacteria (Thomaz and Wetzel 1995). The leaf disks were removed and the remaining sample was incubated for 1 h at 4 °C after the addition of 10 µL of ^{3}H-leucine (99.9 Ci·mmol^{-1}; 1 Ci = 37 Gbc). Bacterial productivity was estimated after accounting for quenching and subtracting the activity of a killed control (Kirchman 1993).

Bacterial biomass was determined on the leaf disks that were preserved in a mixture of 3.7% formaldehyde and 0.1 mol·L^{-1} tetrasodium pyrophosphate (Velji and Albright 1986). Bacterial cells were removed from the leaf disks by sonicating for 30 s on ice. Cells were stained with 4′,6′-diamidino-2-phenylindole and enumerated using epifluorescent microscopy. Cell biovolumes were determined using geometric shapes (Psenner 1993; Wetzel and Likens 2000) and total carbon was estimated by multiplying biovolumes by 5.6×10^{-13} g C·µm^{-3} (Bratbak 1985).

Fungal biomass was estimated from total ergosterol content determined with high performance liquid chromatography (HPLC) analyses (Newell and Fallon 1991; Newell 1995; Wetzel and Likens 2000). Briefly, disks were placed in 10 mL of methanol (HPLC grade) in reflux flasks and refluxed at 80 °C for 30 min in a block heater. Following addition of 5 mL of ultrapure water and 5 mL of pentane (HPLC grade) and mixing by inversion 30 times, the water was removed from the nonpolar pentane fractions. The extraction was repeated 2 additional times. The pentane fractions were evaporated at 30 °C and the lipids were redissolved in 2.0 mL of methanol (HPLC grade) with sonication for 5–10 min. Following filtration, the samples were analyzed by HPLC calibrated against pure standards. Fungal carbon was calculated by multiplying ergosterol concentrations by a ratio of 1 mg of fungal dry mass per 5.5 µg of ergosterol (Gessner and Chauvet 1993).

POA was measured on days 20, 40, and 80. A 3.1-cm diameter disk from each leaf was pulverized in a Dentsply Rinn® model C32003A Wiggle Bug (Dentsply Rinn, Elgin, Illinois, USA) and suspended in 3 mL of 50 mmol·L^{-1} acetate buffer, pH 5.0. This suspension was divided into two 1.5-mL subsamples in glass culture tubes. One subsample received 0.5 mL of a solution of L-3,4-dihydroxyphenylalanine (L-DOPA) in acetate buffer to yield a final concentration of 5 mmol·L^{-1} L-DOPA (Sinsabaugh et al. 1994). The other subsample was used as a blank and received an additional 0.5 mL of acetate buffer. Tubes were capped and incubated at 4 °C for 1 h on a shaker table. Following the incubation period, the tubes were centrifuged and the supernatant was removed and the absorbance was read at 460 nm on a Spectronic® Genesys™ 2 spectrophotometer (Thermo Electron Corporation, Waltham, Massachusetts, USA). POA was calculated in micromoles of substrate converted per hour per gram of leaf dry mass.

Leaf chemistry

Leaf material from days 0 (not incubated in the stream), 20, 40, and 80 was assayed for TPh, CT, BT, and C:N. TPh concentrations were determined using the Folin–Denis assay (Swain and Goldstein 1964) after extraction of 20 mg of sample in 1.5 mL of 70% acetone for 90 min in an ultrasonic water bath maintained at 0 °C. Tannic acid was used as a standard in this assay. Phenol concentrations are therefore based on tannic acid equivalence and are expressed as percent dry mass. CT concentrations were determined from 70% acetone extracts using the acid butanol method (Porter et al. 1986). Concentrations are based on leucocyanidin equivalence and are expressed as percent dry mass.

BT concentrations were determined using a method modified from Terrill et al. (1992). Leaf material that was left behind after extraction with 70% acetone was dried and reweighed. Protein was removed from the material by extracting with a mixture of 1.25 mL of H_2O and 0.75 mL of phenol at 95 °C for 2 h. The samples were centrifuged and the phenol–H_2O mixture was removed. The material was then washed 3 times with ether to remove excess phenol. After drying, condensed tannin concentrations were determined using the acid butanol method. Condensed tannin purified from ambient-CO_2-grown quaking aspen leaves (for the purification procedure see Terrill et al. 1992) was used as a standard in this assay. Serial dilutions of this purified standard dissolved in 70% acetone were dried in a stream of helium before undergoing the acid butanol hydrolysis.

C:N was determined on a Perkin Elmer® 2400 elemental analyzer (Perkin Elmer, Wellesley, Massachusetts). Lignin concentrations were determined on initial samples (day 0) using an acetyl bromide method according to Ilyama and Wallis (1990). Ten milligrams of ground material was extracted in 65 °C water for 1 h followed by filtering onto glass-fiber filters and rinsing with water, ethanol, acetone, and diethyl ether. This step was followed by heating overnight at 50 °C and extracting in a 25% solution of acetyl bromide in acetone at 50 °C. Absorbance of extracts was determined at 280 nm on a Spectronic® Genesys™ 2 spectrophotometer (Thermo Electron Corporation).

Data analysis

All statistical analyses were performed using Systat® version 10 (Systat Software Inc. 2000). Since BT, CT, TPh, and lignin concentrations are expressed as a percentage of leaf

Table 1. Probabilities (*p*) obtained from running two-way ANOVAs on initial chemical parameters for leaf litter produced by three riparian tree species (quaking aspen (*Populus tremuloides*), sugar maple (*Acer saccharum*), and white willow (*Salix alba*)) grown at both ambient (360 parts per million (ppm)) and elevated (720 ppm) concentrations of atmospheric CO_2.

	CO_2 treatment	Species	CO_2 treatment × species
Total soluble phenols	<0.001	<0.001	<0.001
Soluble condensed tannins	0.162	<0.001	0.759
Carbohydrate-bound condensed tannins	0.038	<0.001	0.036
Lignin	0.473	0.181	0.032
C:N	0.019	<0.001	0.118

dry mass, these data were first arcsine ($x^{0.5}$) transformed to improve normality. Estimates of C:N, bacterial carbon, fungal carbon, and POA were log ($x + 1$) transformed to improve normality and normalize variances. A two-way analysis of variance (ANOVA) with species and CO_2 treatment as factors were run on each initial chemical parameter. Three-way ANOVAs with time, species, and CO_2 treatment as factors were used to compare chemistry and microbial data throughout the 80-day in-situ incubation. Correlation analysis was also run between POA and BT, CT, and TPh concentrations in leaf litter collected at all stages of the incubation. Probabilities obtained in the correlation analysis were Bonferroni-corrected to limit overall experimental error rate.

Results

Initial chemical differences

Elevated-CO_2-induced changes in the chemistry of senesced leaf litter were species-specific. There was a significant species × CO_2 interaction ($p = 0.001$; Table 1) with respect to TPh concentration, which indicates that the production of simple phenols, hydrolysable tannins, and condensed tannins under elevated CO_2 was largely species-specific. Maple leaf litter had the highest TPh concentration, while aspen had the lowest (Fig. 1*a*). Within individual species, concentrations were 36% higher in elevated-CO_2-grown maple than in ambient-CO_2-grown maple, a difference of only 12% and 8% being observed between the leaf litter of ambient- and elevated-CO_2-grown aspen and willow, respectively. Although mean CT concentrations wereslightly higher in all three species in the elevated-CO_2 treatments (Fig. 1*b*), species was the only statistically significant factor ($p < 0.001$; Table 1). The BT concentration was highest in aspen and lowest in maple (Fig. 1*c*). The effect of elevated CO_2 on BT was species-specific, with a significant CO_2 × species interaction ($p = 0.036$; Table 1). Concentrations were higher in willow leaves grown under elevated CO_2 than in those grown under ambient CO_2, with leaves grown at elevated CO_2 having nearly twice the BT concentration (Fig. 1*c*). Elevated CO_2 did not produce substantial differences in BT concentration in maple or aspen leaf litter. The response of lignin concentrations to elevated CO_2 was also species-specific, with a significant CO_2 × species interaction ($p = 0.032$; Table 1). Elevated-CO_2-grown aspen leaf litter was 15% higher in lignin than that grown under ambient CO_2 (Fig. 1*d*). Species and CO_2 treatment independently affected C:N, with significant species ($p < 0.001$) and CO_2 treatment ($p = 0.019$) effects with no interaction ($p = 0.118$). C:N was highest in maple followed by willow and then aspen (Fig. 1*e*). Estimates of C:N for maple grown under elevated CO_2 were 25% higher than those for maple grown under ambient CO_2. Estimates for willow and aspen litter were approximately 10% higher for leaves produced under elevated CO_2.

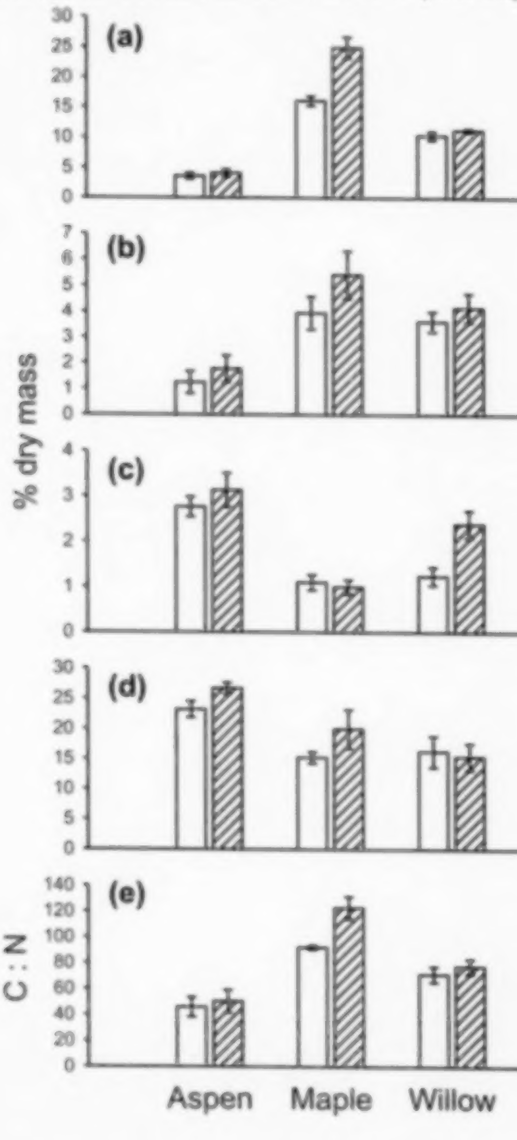
In-situ incubation

Mean daily stream temperatures ranged from –0.1 to 13.1 °C. The average stream temperature for the entire incubation was 4.1 °C. Leaf chemistry changed in all species throughout the incubation period, and in some cases, differences between ambient- and elevated-CO_2-grown leaves became either more apparent or less apparent after incubation in the stream (Fig. 2). Changes in BT through time were largely species-dependent, with a significant time × species interaction ($p < 0.001$; Table 2). BT in aspen leaves decreased slightly throughout the experiment (Fig. 2*a*), while BT in maple increased throughout the first half of the incubation and then leveled off (Fig. 2*b*). BT in willow increased sharply between days 0 and 20 and then declined throughout the remainder of the incubation (Fig. 2*c*). CO_2 treatment was also a significant factor ($p = 0.020$) during the incubation. CO_2-treatment effects were most apparent in willow leaf litter (Fig. 2*c*). Throughout the experiment, with the exception of day 80, BT was highest in elevated-CO_2-grown willow. The greatest difference was on day 20, when elevated-CO_2-grown leaves had almost twice the BT concentration of ambient-CO_2-grown leaves.

CT declined asymptotically in all three species throughout the experiment, although the magnitude of this decline varied among species (Figs. 2*d*–2*f*). Overall, there was a significant time × species interaction ($p < 0.001$; Table 2). Although no initial CO_2 effects on CT were observed for these three species, a slight but significant CO_2 effect ($p = 0.020$) was observed during the incubation (Fig. 2*e*). Similarly, TPh declined in all three species over the course of the experiment (Figs. 2*g*–2*i*), with a significant time × species interaction ($p < 0.001$; Table 2). There was also a significant effect ($p = 0.002$) of elevated-CO_2-grown leaves on TPh during the incubation.

C:N declined nearly linearly from initial values throughout the incubation (Figs. 2*j*–2*l*). A significant time × species interaction ($p < 0.038$; Table 2) was observed along with a highly significant CO_2 effect ($p < 0.001$). Differences in C:N between the leaf litter of ambient- and elevated-CO_2-grown

Fig. 1. Initial chemistry values (mean ± 1 standard error) including total soluble phenols (*a*), soluble condensed tannins (*b*), carbohydrate-bound condensed tannins (*c*), lignin (*d*), and the ratio of organic carbon to nitrogen, C:N (*e*), of naturally senesced leaf litter collected from three riparian tree species (quaking aspen (*Populus tremuloides*), sugar maple (*Acer saccharum*), and white willow (*Salix alba*) grown at both ambient (360 parts per million (ppm); open bars) and elevated (720 ppm; hatched bars) concentrations of atmospheric CO_2.



trees were most apparent initially in maple (Fig. 2*k*) and at the midpoint of the incubation in willow (Fig. 2*l*).

Bacterial biomass developing on leaf litter of all three species increased through the first 40 days of in-situ incubation and then plateaued (Figs. 3*a*–3*c*). All three variables (time, species, and CO_2 treatment) independently produced significant (Table 2) effects on bacterial biomass. Mean bacterial biomass was consistently lower on tissues that had been grown at elevated CO_2 in all species, but more so on the aspen litter (Fig. 3*a*). The productivity of attached bacteria on leaves of all species increased throughout the incubation (Figs. 3*d*–3*f*), producing a significant time effect ($p <$ 0.001; Table 2). However, there were no species or CO_2 treatment effects (Table 2). Fungal biomass remained low on all species throughout the first 40 days of the experiment, and then more than doubled during the final 40 days (Figs. 3*g*–3*i*), producing a significant time × species interaction (p = 0.018; Table 2). However, whether leaves were grown at ambient or elevated CO_2 had no significant effect on fungal biomass (p = 0.786).

POA was generally higher on the aspen leaves than on leaves of the other two species (Figs. 3*j*–3*l*), and increased throughout the incubation (Fig. 3*j*). POA on maple leaves declined slightly between days 20 and 40 and then increased slightly between days 40 and 80 (Fig. 3*k*). POA on willow leaves remained constant between days 20 and 40 and then increased between days 40 and 80 (Fig. 3*l*). There were significant time × species ($p < 0.001$) and CO_2 × time (p = 0.027) interactions with respect to POA (Table 2). The effect of elevated CO_2 was most apparent in willow, where ambient-CO_2-grown leaves displayed higher POA throughout the incubation (Fig. 3*l*). In addition, POA on willow leaf litter was negatively correlated with BT ($r = -0.517$, $p <$ 0.001), CT ($r = -0.349$, $p = 0.001$), and TPh ($r = -0.316$, $p =$ 0.003).

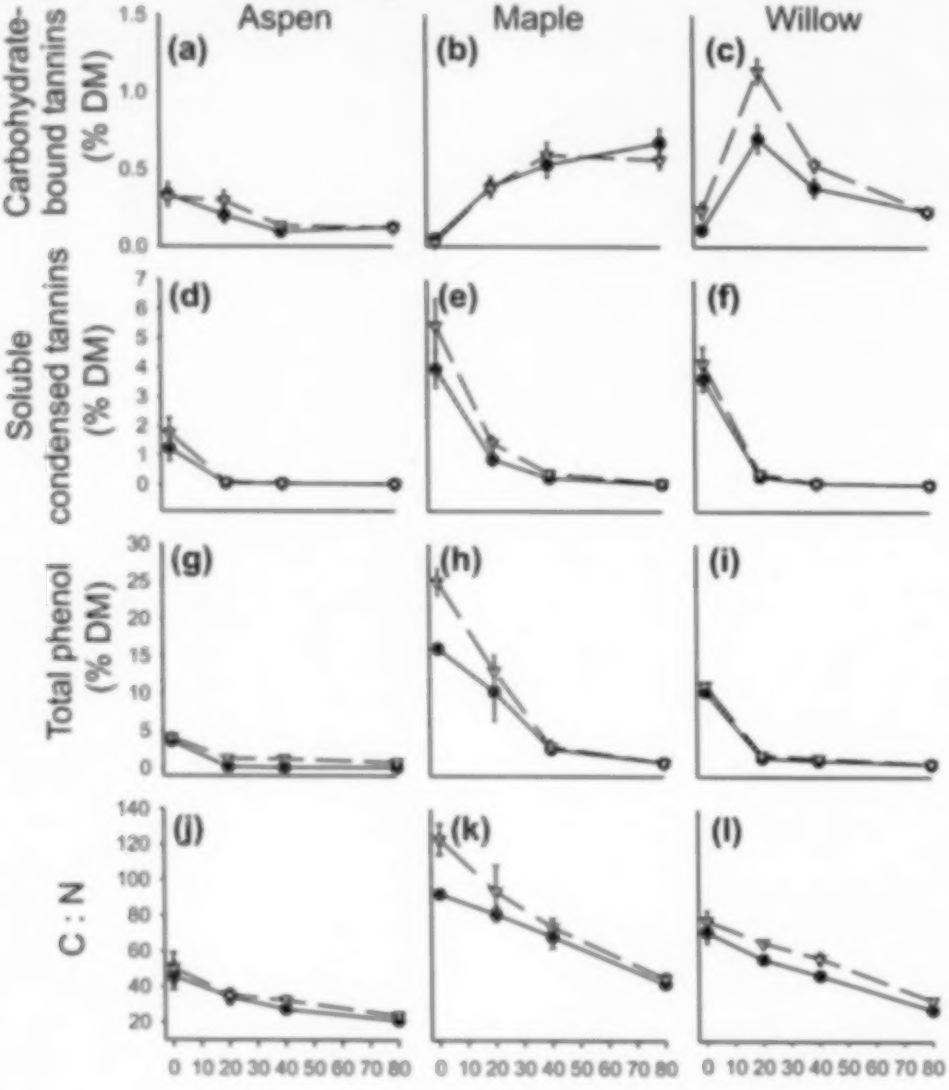
Discussion

Litter chemistry

Alterations of leaf-litter chemistry resulting from growth under elevated CO_2 was species-specific, but in all species favored production of carbon-rich compounds such as lignin, tannins, and other phenols, which were maintained after leaf senescence. Lindroth et al. (1993) demonstrated a similar species-specific response to elevated CO_2, with sugar maple producing more phenolic defense compounds and quaking aspen producing more structural carbohydrates. King et al. (2001*a*, 2001*b*) found similar differences between sugar maple and quaking aspen and speculated that since quaking aspen is a fast-growing early-successional species, it will allocate more carbon to growth, whereas sugar maple, a slow-growing late-successional species, will invest more carbon into secondary metabolites.

Since growth under elevated CO_2 caused an increase in BT but not a substantial increase in CT in the willow leaf litter, future studies into the effects of elevated CO_2 on plant phenolic compounds should assay for both types of condensed tannins because both show potentially important ecological responses. Most studies into the effects of elevated CO_2 on leaf-litter decomposition only measure the fraction of condensed tannins that is soluble in either acetone or methanol (e.g., King et al. 2001*a*, 2001*b*; Veteli et al. 2002). Even though soluble phenols may have the potential to inhibit leaf-litter decomposition in aquatic systems (Stout 1989), concentrations generally are not highly correlated

Fig. 2. Leaf litter chemistry values (mean ± 1 standard error) including total soluble phenols (*a*–*c*), soluble condensed tannins (*d*–*f*), carbohydrate-bound condensed tannins (*g*–*i*), and C:N (*j*–*l*) throughout an 80-day in-stream incubation of three common riparian tree species (quaking aspen, sugar maple, and white willow) grown at both ambient (360 parts per million (ppm); solid lines) and elevated (720 ppm; broken lines) concentrations of atmospheric CO_2 (DM is dry mass).



with decomposition rates (Ostrofsky 1993; Gessner and Chauvet 1994; Campbell and Fuchshuber 1995). The lack of correlation between soluble phenol concentrations and decomposition rates may be partially due to leaching. For example, Benstead (1996), working in a tropical stream, showed that soluble phenols are leached out of leaf litter shortly after immersion. Rier et al. (2002), working with ambient- and elevated-CO_2-grown aspen leaves demonstrated that this fraction is rapidly leached, after immersion in water for only 24–48 h. In the current study, BT persisted in both maple and willow throughout the 80-day in-stream incubation. The difference between ambient- and elevated-CO_2-produced willow leaf litter persisted throughout much of the incubation and may help to explain the persistence of a small difference in C:N between the two treatments.

Microbial response

Despite detectable changes in leaf-litter chemistry following growth under elevated CO_2, differences in rates of colonization by fungi and bacteria in the stream incubations

Table 2. Probabilities (*p*) obtained from running three-way ANOVAs on leaf chemistry and microbial parameters for leaf litter produced by three riparian tree species (quaking aspen, sugar maple, and white willow) grown at both ambient (360 parts per million (ppm) and elevated (720 ppm) concentrations of atmospheric CO_2 during an 80-day in-stream incubation.

	CO_2 treatment	Species	Time	Time × species	CO_2 treatment × time	CO_2 treatment × species	CO_2 treatment × time × species
Leaf chemistry							
Total soluble phenols	0.011	<0.001	<0.001	<0.001	0.077	0.284	0.136
Soluble condensed tannins	0.002	<0.001	<0.001	<0.001	0.156	0.097	0.922
Carbohydrate-bound condensed tannins	0.020	<0.001	<0.001	<0.001	0.149	0.143	0.467
C:N	0.001	<0.001	<0.001	0.038	0.883	0.569	0.839
Microbial parameter							
Bacterial biomass	0.013	<0.001	<0.001	0.967	0.172	0.515	0.823
Bacterial productivity	0.609	0.750	<0.001	0.594	0.790	0.693	0.944
Fungal biomass	0.876	0.458	<0.001	0.018	0.564	0.270	0.221
Phenol oxidase activity	0.786	<0.001	<0.001	<0.001	0.027	0.105	0.518

were small. Initially, the largest chemical difference was a 36% increase in TPh among maple leaves grown at elevated CO_2. The difference may have produced the observed differences in bacterial biomass in maple after the first 20 days of incubation. However, this difference was no longer apparent after 20 days and likely resulted from leaching of soluble phenols from the leaves within several days of immersion.

The greater persistence of higher bacterial biomass on ambient-CO_2-produced aspen litter than on elevated-CO_2-produced aspen litter likely resulted from changes induced by elevated CO_2 that were not associated with soluble phenols, which appeared to be readily leached. It is possible that the initially higher lignin content of the elevated-CO_2-produced aspen litter might have persisted throughout the incubation, preventing bacteria from utilizing the more labile carbon substrates associated with the leaf matrix.

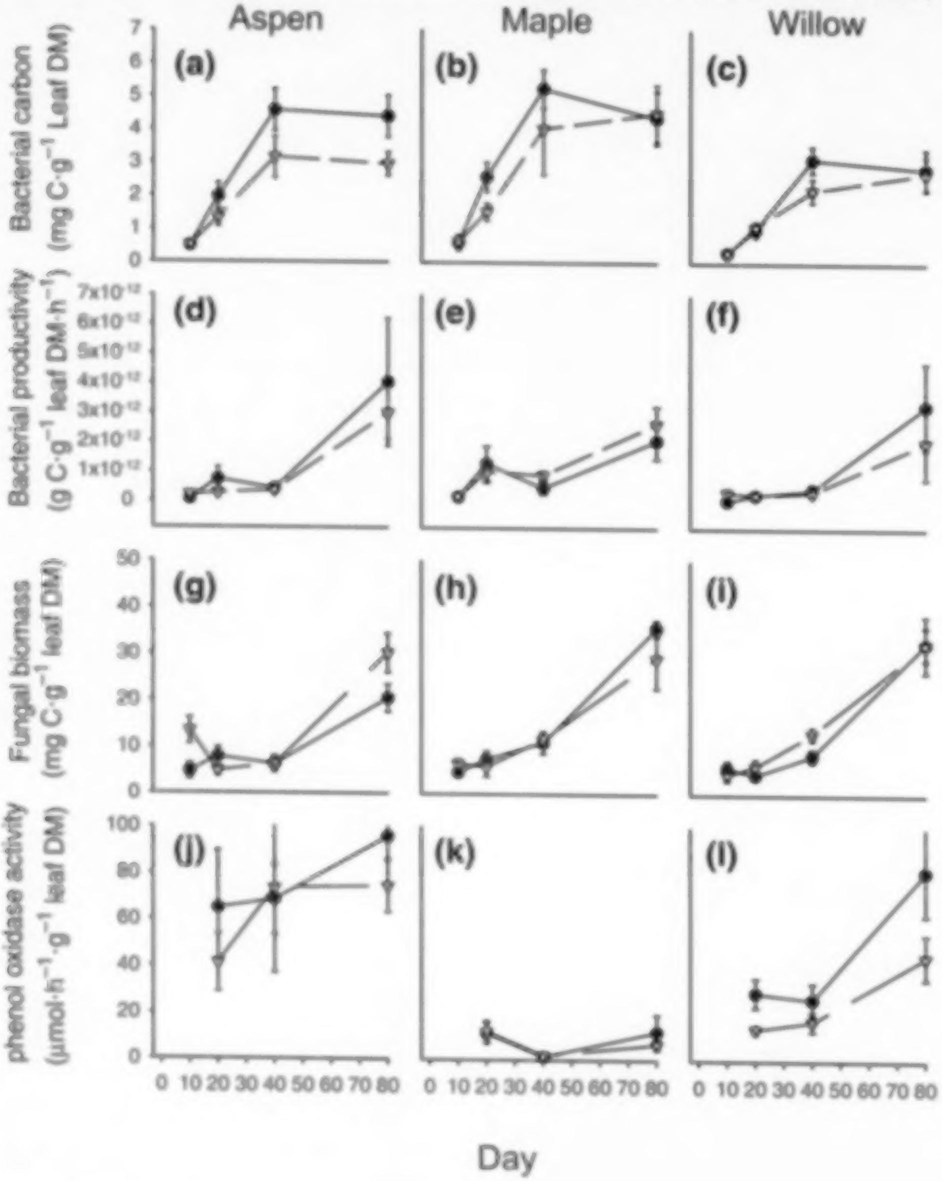
Two methodological limitations my have contributed to an inability to detect treatment effects on bacterial productivity and fungal biomass. Because the bacteria were first removed from the surface of the leaf litter using sonication, it is likely that bacterial productivity in suspension from that on the surface of the leaf litter. It is also possible that the physical disruption associated with sonication metabolically altered the bacteria, although this limitation has been found to be minor (cf. Thomaz and Wetzel 1995). Another possible limitation is the stream temperature at the beginning of the experiment. This experiment was performed during the winter, when the temperature of the stream often approached freezing. It is possible that bacterial productivity and fungal development during the first half of the experiment were more limited by temperature than by the presence or absence of various chemical substrates. This might help to explain the overall low fungal biomass throughout this experiment relative to other studies (e.g., Gessner and Chauvet 1994).

The observed differences in POA between the litter of ambient- and elevated-CO_2-grown willows may have resulted from the higher concentrations of polyphenolic compounds in the elevated-CO_2-grown litter. We originally hypothesized that higher concentrations of polyphenolic compounds — associated with growth under elevated CO_2 — would stimulate the production of phenol oxidases by the microbial community in order to utilize these compounds. However, the opposite trend was observed, with highly significant negative correlations between BT, CT, and TPh and POA. It is possible that POA was inhibited by condensed tannins that were bound to carbohydrates, CT that were in the process of leaching from the litter, or other soluble phenolic compounds. Enzyme inhibition by these compounds has been known for many decades (e.g., Haslam 1988) and it has been widely observed that tannins and other phenolic compounds complex many different membrane-bound and extracellular enzymes by noncompetitive inhibition (e.g., Stewart and Wetzel 1982; Wetzel 1991, 1993).

A number of terrestrial studies have not shown appreciable differences in leaf-litter decomposition among plants grown at ambient and elevated CO_2. Norby et al. (2001), using meta-analysis to examine a large array of published and unpublished data, demonstrated that although growth under elevated CO_2 does affect leaf-litter chemistry, these effects do not result in slower rates of decomposition. In addition, in studies with quaking aspen and sugar maple, initial chemical differences were not correlated with rates of leaf-litter decomposition (King et al. 2001*a*, 2001*b*). Furthermore, Finzi and Schlesinger (2002) showed in a free-air CO_2-enrichment experiment that species composition was more important in explaining litter decomposition rates than growth under elevated CO_2.

The current study and other in-situ studies (e.g., Rier et al. 2002; Tuchman et al. 2003*b*) have shown only subtle effects of enriched-CO_2-induced alterations to leaf-litter chemistry on decomposition and microbial activity. Other studies have demonstrated marked negative effects of altered chemistry on consumption by and growth and development of aquatic invertebrate detritivores utilizing these materials and their attached communities (Tuchman et al. 2002, 2003*a*, 2003*b*). The discrepancies among the observed decomposition rates, microbial activities, and detritivore responses might be associated with variable leaching rates of inhibitory compounds from the leaf tissues upon immersion. Relatively short incubations, such as 2 weeks, were clearly adequate to preserve inhibitory compounds in order to suppress biological development of colonizing organisms. With longer incubations in

Fig. 3. Microbial activity (mean ± 1 standard error), including bacterial carbon (*a–c*), bacterial productivity (*d–f*), fungal biomass (*g–i*), and phenol oxidase activity (*j–l*), on leaf litter from three riparian tree species (quaking aspen, sugar maple, and white willow) grown at both ambient (360 parts per million (ppm); solid lines) and elevated (720 ppm; broken lines) concentrations of atmospheric CO_2.



flowing waters, inhibitory-compound reductions could allow the development of microbial communities and enhanced invertebrate consumption (cf. Golladay et al. 1983). These differences are supported by studies of macroinvertebrate colonization on aspen leaf litter, produced under ambient and elevated CO_2, which was incubated in a stream for 120 days and showed increasingly disparate results with longer incubation periods (Tuchman et al. 2003*b*). Other species will surely vary in their responses to growth at elevated CO_2. For example, the present study showed that differences in BT carbohydrate-bound tannins between ambient- and elevated-CO_2-produced willow leaf litter were retained for at least 40 days in the stream. Negative effects on colonizing microbial and consuming detritivores would likely extend for considerable periods of time.

In conclusion, the impact of altered leaf chemistry on riparian trees grown under elevated CO_2 is clearly variable, as this leaf litter is immersed and leached in rapidly flowing streams. However, other mechanisms could indirectly impact forest streams. For example, this study did not address the fraction of the dissolved organic matter (DOM) pool that is leached from the litter either directly in the stream or through soil–water pathways. DOM constitutes an important fraction of allochthonous carbon entering most stream ecosystems (Wetzel 2001). Since phenolic defense compounds are likely produced in excess by a number of riparian tree species under elevated CO_2 and appear to be readily leached, there is a potential for marked shifts in the recalcitrance of the DOM pool as atmospheric CO_2 increases. This study also did not address potential increases in leaf-litter inputs to forested streams. Trees often produce more leaf litter under elevated CO_2 (e.g., Finzi and Schlesinger 2002), which could influence stream carbon fluxes and stoichiometry. Our results imply that such effects will depend on the species composition of the vegetation and on the kinetics of leaf-litter leaching and decomposition.

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Resmoltification in wild, anadromous Arctic char (*Salvelinus alpinus*): a survey of osmoregulatory, metabolic, and endocrine changes preceding annual seawater migration

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Abstract: Anadromous Arctic char (*Salvelinus alpinus*) migrate to seawater every summer for feeding and spend the rest of the year overwintering in fresh water. We investigated whether annual seawater migration is preceded by changes in hyposmoregulatory capacity, intermediary metabolism, and the plasma levels of hormones known to play a role in salmonid seawater preadaptation (smoltification). Wild, anadromous Arctic char were sampled in their overwintering lake in April and May and during the period of downstream migration in June. Our results demonstrate a fourfold seasonal increase in gill Na^+/K^+ ATPase activity, with maximum levels in descending migrants that also displayed prime hyposmoregulatory capacity. Hepatosomatic index and activities of key liver enzymes also increased during spring, indicating a general increase in liver metabolic capacity. These changes were accompanied by increased plasma cortisol and thyroxine levels, decreased plasma growth hormone levels, and unchanged plasma levels of insulin-like growth factor-I and 3,5,3′-triiodothyronine. Our results indicate that wild, anadromous Arctic char resmoltify every spring, and this involves a shift from an energy-conserving overwintering state to a state where they become osmotically and metabolically prepared for their annual feeding migration to seawater.

Résumé : Les ombles chevaliers (*Salvelinus alpinus)* anadromes migrent vers l'eau de mer tous les étés pour se nourrir et passent le reste de l'année à hiverner en eau douce. Nous avons vérifié si la migration annuelle vers la mer est précédée de changements dans la capacité hyposmorégulatrice, le métabolisme intermédiaire et les concentrations plasmatiques d'hormones qui jouent un rôle dans la préadaptation des salmonidés à l'eau salée (transformation en saumoneau). Nous avons prélevé des ombles chevaliers anadromes sauvages en avril et en mai dans un lac où elles passent l'hiver et en juin durant la période de la migration vers l'aval. Nos résultats montrent une augmentation saisonnière par un facteur de 4 de l'activité de la Na^+/K^+ adénosine triphosphatase (ATPase) dans la branchie, avec une activité maximale observée chez les poissons en migration vers l'aval; ceux-ci possèdent aussi une importante capacité hyposmorégulatrice. L'indice hépatosomatique et l'activité des enzymes importants du foie augmentent aussi au cours du printemps, ce qui indique un accroissement général de l'activité métabolique du foie. Ces changements s'accompagnent d'une augmentation des concentrations plasmatiques de cortisol et de thyroxine, d'une diminution des concentrations de l'hormone de croissance et du maintien des concentrations du facteur de croissance I analogue à l'insuline et de la 3,5,3′-triiodothyronine. Nos résultats indiquent que les ombles chevaliers sauvages anadromes se transforment en saumoneaux chaque printemps, un processus qui implique une transition d'un état de conservation de l'énergie pendant l'hiver à un état dans lequel elles se préparent osmotiquement et métaboliquement à leur migration annuelle vers la mer pour s'alimenter.

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Introduction

Before establishing an anadromous life history, juvenile salmonid fish may undergo a series of physiological, biochemical, and behavioural transformations that preadapt the fish for marine residency, collectively known as parr–smolt transformation, smoltification, or smolting (see reviews by Hoar 1988; McCormick et al. 1998). Smoltification is stimulated by increased daylength in spring and is orchestrated by hormones such as cortisol, growth hormone (GH), insulin-like growth factor-I (IGF-I), and the thyroid hormones 3,5,3′-triiodothyronine (T_3) and thyroxine (T_4). During this developmental process, territorial bottom-dwelling parr transform into schooling, streamlined, silvery smolts and develop hyposmoregulatory capacity and migrate to the sea.

The seawater (SW) preadaptive properties attained through smoltification are lost if smolts are prevented from establishing an anadromous life history (Hoar 1988). In a recent laboratory study, however, it was demonstrated that Atlantic salmon (*Salmo salar*) smolts that were retained in fresh water (FW) following smoltification were capable of smolting again the following year (Shrimpton et al. 2000). Although all species of Pacific salmon (belonging to *Oncorhynchus* sp.) are semelparous and perform a single seaward migration only, a life history involving repeated SW migrations naturally occurs in the anadromous Pacific trout (*Oncorhynchus* sp.) and within the anadromous species of the genera *Salmo*, *Salvelinus*, and *Hucho* (Rounsefell 1958; Stearley 1992; Klemetsen et al. 2003). Despite this widespread occurrence of repeated SW migration among salmonids, it is currently not known whether or not fish that have been allowed to establish an anadromous life history smolt again prior to subsequent SW migrations.

The salvelinid Arctic char (*Salvelinus alpinus*) has a circumpolar distribution and is the world's northernmost living FW fish. Following parr–smolt transformation and first SW migration, anadromous Arctic char spend only 1–2 months feeding in SW during summer after which both immature and mature char return to FW for overwintering (Nordeng 1983; Klemetsen et al. 2003). In SW, food availability is high, and anadromous Arctic char may double their body mass and increase their storage lipid depots severalfold (Jørgensen et al. 1997). In FW, food availability is poor, and anadromous individuals experience prolonged fasting and mobilization of endogenous energy substrates stored during summer (Boivin and Power 1990; Jørgensen et al. 1997). As a result, anadromous Arctic char display reduced nutritional status when returning to SW each summer (Jørgensen et al. 1997).

Studies have shown that downstream migrants of anadromous Arctic char possess a predeveloped hyposmoregulatory capacity (e.g., Nilssen et al. 1997). Laboratory studies have further demonstrated that development of hyposmoregulatory capacity and a corresponding increase in gill Na^+/K^+ ATPase activity are stimulated by increased daylength in spring (Arnesen et al. 1992; Johnsen et al. 2000). However, very little is known about the endocrine and metabolic adjustments associated with SW preadaptation in Arctic char (see Aas-Hansen et al. 2003). Further, general knowledge on the hormonal regulation of energy metabolism and smoltification is largely based on laboratory studies using hatchery-reared fish lacking the entrainment of their natural environment and with a nutritional status differing considerably from that of their wild counterparts. Such differences may well affect the integrated regulation of the smoltification processes, especially since the hormones regulating smoltification also regulate growth and metabolism (Dickhoff et al. 1997). In this respect, it is particularly interesting to study repeat migrants of wild, anadromous Arctic char, since possible SW preadaptive processes are implemented during a period of prolonged natural fasting. Further, the integration of smoltification processes may be particularly advantageous to study in high-latitude systems where the animal's physiology is strictly coordinated with the pronounced seasonal variation of the environment.

Consequently, the aims of the present study were to (*i*) investigate osmoregulatory, metabolic, and endocrine adjustments associated with seasonal SW migration in wild, anadromous Arctic char and (*ii*) evaluate whether smoltification processes are resumed prior to seaward migration in these repeat migrants. To this end, we have sampled wild, overwintering anadromous Arctic char in their natural habitat on three occasions during late winter to early summer. The last sampling was performed on descending migrants and also included a SW challenge test (SWT).

Methods

Study area and design

The study was performed in Laksvatn, a shallow, 0.8-km^2 oligotrophic lake in northern Norway (69°N). It is situated 3 m above sea level and has a 600-m outlet stream without falls. The Laksvatn Arctic char population displays a high degree of anadromy with only a small fraction of nonanadromous adults (Kristoffersen et al. 1994). Wild Arctic char were sampled either directly in the overwintering lake or during downstream migration in the Laksvatn outlet river. When sampling in the lake, Arctic char were caught by hook and line from the ice with a reflective jig and a baited hook on 8 April ($n = 5$) and 10 May ($n = 7$). During downstream migration on 1 June, descending Arctic char ($n = 7$) were caught with a fish trap temporarily mounted in the Laksvatn outlet river approximately 200 m upstream of the estuary. The fish trap was continuously monitored and did not seem to influence the behaviour of the fish. In addition, 32 downstream migrants caught in the fish trap during the period 28–31 May were transferred to a SW tank on 31 May and sampled as part of a standardized SWT during the period 31 May – 2 June.

Sampling procedures, FW

Captured fish were killed by concussion and body mass (BM) and fork length (FL) were recorded and used for later calculation of condition factor (Kf). Blood, gill filaments, and liver were sampled and sex and maturity status (visual inspection of gonads) were routinely registered. Presence of the marine parasites *Anisakis simplex* (a nematode) and metacercariae of the digenean *Cryptocotyle lingua* and indices of previous infection (bite marks) by the ectoparasitic marine crustacean *Lepeophtheirus salmonis* were also registered and used as indicators of anadromous life history (cf. Kristoffersen et al. 1994). Blood was sampled from the caudal vasculature by lithium-heparinized vacutainers (Vacutainer®, BD Diagnostics, Franklin Lakes, New Jersey) and kept on ice until plasma was obtained by centrifugation (2700*g* for 8 min at 2 °C). The time from fish capture to collection of blood never exceeded 3 min. Plasma aliquots were stored frozen (–70 °C) for later analyses of plasma cortisol, GH, IGF-I, T_3, and T_4 concentrations as well as plasma glucose and lactate concentrations. In addition, plasma osmolality and chloride concentration were also analysed in descending migrants to compare the levels found in fish subjected to SWT (see separate section below). Approximately 10 mg of gill filaments was dissected from the second gill arch on the left side by fine-tip forceps, put into 100 µL of ice-cold isotonic SEI solution (0.3 mol·L^{-1} sucrose, 0.02 mol·L^{-1} EDTA, and 0.1 mol·L^{-1} imidazole), frozen in liquid nitrogen, and stored at –70 °C for later analysis of gill Na^+/K^+ ATPase activity. The whole liver (gallbladder carefully excised) was weighed, immediately frozen in liquid nitrogen, and stored at –70 °C for later analyses of glycogen content, glucose concentration, and metabolic enzyme activities. Sacrificed fish were stored at –20 °C for later determination of age, presence of marine parasites, and stomach fullness. Water temperature was not registered during ice-fishing (i.e., on 8 April and 10 May), but the lake was completely covered with thick ice on both these samplings. Ice breakup (and thus the vernal temperature upheaval) did not occur until late May, suggesting a water temperature between 0 and 4 °C on these samplings. During downstream migration on 1 June, river temperature was approximately 7 °C but fluctuated between 4 and 8 °C in the period 31 May – 2 June. All FW samplings were performed in the afternoon (between 1200 and 1600).

SWT

Approximately 50 descending migrants that were captured in the fish trap in the period 28–31 May were transferred into a mesh cage temporarily placed in the river. On 31 May, an SWT was initiated by transferring 32 of these fish (selection aimed at minimizing size variation) into a 1000-L plastic tank filled with oxygenated SW (SWT tank). The SWT tank was placed partly submerged in the river, and renewal of SW was ensured by means of continuous inflow (1.5 L·min^{-1}) from another 1000-L tank that was refilled with SW from the fjord twice a day. The temperature of the SWT tank varied from 7 °C at the onset of the SWT to 11 °C 48 h thereafter, whereas the oxygen saturation was kept above 70%. The salinity was stable at 34‰–35‰. Fish were sampled following 3, 6, 24, and 48 h of SW exposure. On each sampling, fish (n = 8) were quickly and carefully dipnetted and killed (as described above), after which BM, FL, sex, maturation, and infection with marine parasites were recorded. Blood was sampled and treated as described above for FW-sampled fish and used for later analyses of plasma osmolality and chloride concentration.

Analyses and calculations

The Kf was calculated from the formula Kf = BM × 100 × FL^{-3} (with BM in grams and FL in centimetres). Fish age was determined by using the otoliths (Kristoffersen and Klemetsen 1991). Stomach fullness was determined by visual inspection. Infection (and indices of previous infection) with marine parasites was assessed as described by Kristoffersen et al. (1994). Gill Na^+/K^+ ATPase (EC 3.6.1.3) activity was analysed by a standard microassay procedure (Schrock et al. 1994).

Plasma cortisol levels were measured in diethyl ether extracted plasma using a commercially available radioimmunoassay (RIA) kit (Neogene®, Lexington, Kentucky) that was validated for Arctic char (J. Wolkers, Norwegian College of Fishery Science, University of Tromsø, N-9037 Tromsø, Norway, unpublished data) towards a cortisol RIA assay established in our laboratory (Jørgensen et al. 2002). Plasma GH levels were measured using a noncompetitive enzyme-linked immunosorbent assay recently validated for Arctic char (Cameron et al. 2002). Plasma T_3 and T_4 concentrations were analysed using commercially available RIA kits (Amerlex-M®; Trinity Biotech plc., Wicklow, Ireland) previously described and validated for use with various salmonid species, including brook trout (*Salvelinus fontinalis*) (Leatherland and Farbridge 1992; Vijayan and Leatherland 1992). Total IGF-I levels were measured in acid–ethanol-extracted plasma using a commercially available RIA kit (GroPep Ltd., Adelaide, Australia) validated for salmonids (Shimizu et al. 2000). Plasma glucose and lactate concentrations were measured spectrophotometrically using commercially available enzymatic kits (Sigma Diagnostics Inc., St. Louis, Missouri). Plasma osmolality was analysed with a Fiske One-Ten osmometer (Fiske Associates, Norwood, Massachusetts), and plasma chloride concentration was measured with a Corning 925 chloride titrator (CIBA Corning Diagnostics, Essex, England).

Hepatosomatic index (HSI) was calculated from the formula HSI = liver mass × 100 × BM^{-1}, where liver mass and BM are in grams. Liver protein concentration was measured using the bichinchoninic acid method with bovine serum albumin as the standard (Sigma Diagnostics Inc.). Liver glycogen content was determined by liver glucose measurements before and after amyloglucosidase hydrolysis (Keppler and Decker 1974) and expressed as glucosylic units per 100-g fish (similar to that described for enzyme activities, see below). For liver enzyme activity measurements, the liver was homogenized (Ultra Turrax; IKA Works, Wilmington, North Carolina) followed by sonication (Microson, New York) in a 1:10 (*w/v*) buffer containing 21 mmol·L^{-1} Na_2HPO_4, 5 mmol·L^{-1} 2-mercaptoethanol, 0.5 mmol·L^{-1} EDTA, 0.2% bovine serum albumin, 10 µg aprotinin·mL^{-1}, and 50% (*v/v*) glycerol and pH adjusted to 7.4. Liver enzyme activities were measured in 50 mmol·L^{-1} imidazole-buffered (pH 7.4) homogenate in a final volume of 250 µL at 22 °C by continuous spectrophotometry at 340 nm on a microplate reader (ThermoMax; Molecular Devices Corp., Palo Alto, California). The enzyme activities determined included alanine aminotransferase (AlaAT) (EC 2.6.1.2), aspartate aminotransferase (AspAT) (EC 2.6.1.1), glucose-6-phosphate dehydrogenase (G6PDH) (EC 1.1.1.49), 3-hydroxyacyl-coenzyme A dehydrogenase (HOAD) (EC 1.1.1.35), lactate dehydrogenase (LDH) (EC 1.1.1.27), phosphoenolpyruvate carboxykinase (PEPCK) (EC 4.1.1.32), and pyruvate kinase (PK) (EC 2.7.1.40). The following assay conditions were used. AlaAT: 0.12 mmol·L^{-1} NADH, 200 mmol·L^{-1} L-alanine, 0.025 mmol·L^{-1} pyridoxal 5-phosphate, 10.5 mmol·L^{-1} α-ketoglutarate, and 3 units of LDH; AspAT: 40 mmol·L^{-1}

Table 1. Condition factor (Kf), plasma glucose and lactate levels, hepatosomatic index (HSI), liver glycogen content and glucose concentration, and activities of the liver enzymes alanine aminotransferase (AlaAT), aspartate aminotransferase (AspAT), glucose-6-phosphate dehydrogenase (G6PDH), hydroxyacyl-coenzyme A dehydrogenase (HOAD), lactate dehydrogenase (LDH), phosphoenolpyruvate carboxykinase (PEPCK), and pyruvate kinase (PK) of wild, anadromous Arctic char (*Salvelinus alpinus*) sampled during ice-fishing on 8 April ($n = 5$) and 10 May ($n = 7$) and during downstream migration on 1 June ($n = 7$).

	8 April	10 May	1 June
Kf	0.84±0.03a	0.79±0.02a	0.77±0.01a
Plasma glucose (mmol·L^{-1})	3.08±0.25a	5.30±0.13b	5.94±0.32b
Plasma lactate (mmol·L^{-1})	0.68±0.25a	1.17±0.31a	1.14±0.16a
HSI (liver weight·100-g fish^{-1})	0.74±0.03a	0.95±0.04b	0.91±0.03b
Liver glycogen (µmol glucosylic units·100-g fish^{-1})	79.0±9.6a	43.9±6.8b	33.7±6.4b
Liver glucose (µmol·100-g fish^{-1})	23.7±5.9ab	31.0±6.1a	11.4±3.9b
Liver AlaAT (U·100-g fish^{-1})	22.1±7.1a	63.6±7.7b	70.3±10.2b
Liver AspAT (U·100-g fish^{-1})	15.9±2.5a	32.3±2.5b	28.6±3.3b
Liver G6PDH (U·100-g fish^{-1})	6.9±1.7a	13.6±1.1b	12.9±1.7b
Liver HOAD (U·100-g fish^{-1})	0.43±0.04a	0.44±0.04a	0.58±0.02b
Liver LDH (U·100-g fish^{-1})	116.9±8.1a	175.7±9.6b	169.0±10.1b
Liver PEPCK (U·100-g fish^{-1})	0.73±0.22a	1.90±0.19b	2.00±0.14b
Liver PK (U·100-g fish^{-1})	13.0±1.2a	22.8±2.2ab	29.3±3.6b

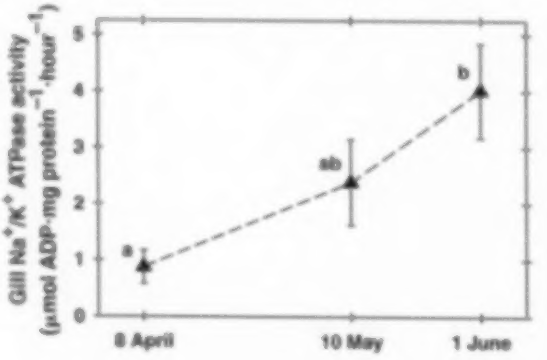
Note: Values are means ± SE of the mean. Different letters indicate a significant difference in this parameter between samplings (ANOVA, $p < 0.05$). The values for liver parameters have been standardized to a 100-g fish. U, micromoles of substrate consumed or product liberated per minute.

aspartate, 7 mmol·L^{-1} α-ketoglutarate, 0.025 mmol·L^{-1} pyridoxal 5-phosphate, 0.12 mmol·L^{-1} NADH, and 2 units of malate dehydrogenase (EC 1.1.1.37); G6PDH: 7 mmol·L^{-1} $MgCl_2$, 1 mmol·L^{-1} glucose-6-phosphate, and 0.4 mmol·L^{-1} β-NADP; HOAD: 0.05 mmol·L^{-1} acetoacetyl-coenzyme A, and 0.05 mmol·L^{-1} NADH; LDH: 1 mmol·L^{-1} pyruvate and 0.12 mmol·L^{-1} NADH; PEPCK: 20 mmol·L^{-1} $NaHCO_3$, 1 mmol·L^{-1} $MnCl_2$, 0.5 mmol·L^{-1} phosphoenolpyruvate (PEP), 0.2 mmol·L^{-1} deoxyguanosine diphosphate, and 0.12 mmol·L^{-1} NADH; PK: 30 mmol·L^{-1} KCl, 10 mmol·L^{-1} $MgCl_2$, 2.5 mmol·L^{-1} ADP, 0.12 mmol·L^{-1} NADH, 5 units of LDH, and 2.5 mmol·L^{-1} PEP. The enzyme activities of each homogenized sample was first calculated as micromoles of substrate consumed or product liberated per minute (U) per milligram of protein in the homogenate. To assess the relative importance at the whole-animal level, we multiplied enzyme activities in the homogenates by the actual liver protein concentration (expressed as milligrams of protein per gram of liver) and by the HSI to yield total liver enzyme activity standardized to a 100-g fish (Bastrop et al. 1992).

Statistics

Except for plasma lactate and liver HOAD activity in April and plasma cortisol and liver glycogen content in June, all data were normally distributed (one-sample Kolmogorov–Smirnov, Lilliefors distribution). Possible effects of time for normally distributed data were tested by a one-way analysis of variance (ANOVA), and a Tukey post hoc test was used to reveal time differences. For the parameters including data that were not normally distributed (i.e., plasma cortisol and lactate, HOAD activity, and liver glycogen), the nonparametric Kruskal–Wallis one-way ANOVA test was used to reveal possible differences between sampling dates. A Student's *t* test was used to compare BM, FL, Kf, plasma osmolality, and chloride concentration between descending migrants sampled in FW and those sampled during the SWT. A probability level of 5% was considered significant in all tests. Data are presented as mean ± SE of the mean.

Fig. 1. Gill Na^+/K^+ ATPase activity of wild anadromous Arctic char (*Salvelinus alpinus*) sampled during ice-fishing on 8 April ($n = 5$) and 10 May ($n = 7$) and during downstream migration on 1 June ($n = 7$). Values are means ± SE of the mean. Different letters indicate a significant difference between samplings (ANOVA, $p < 0.05$).

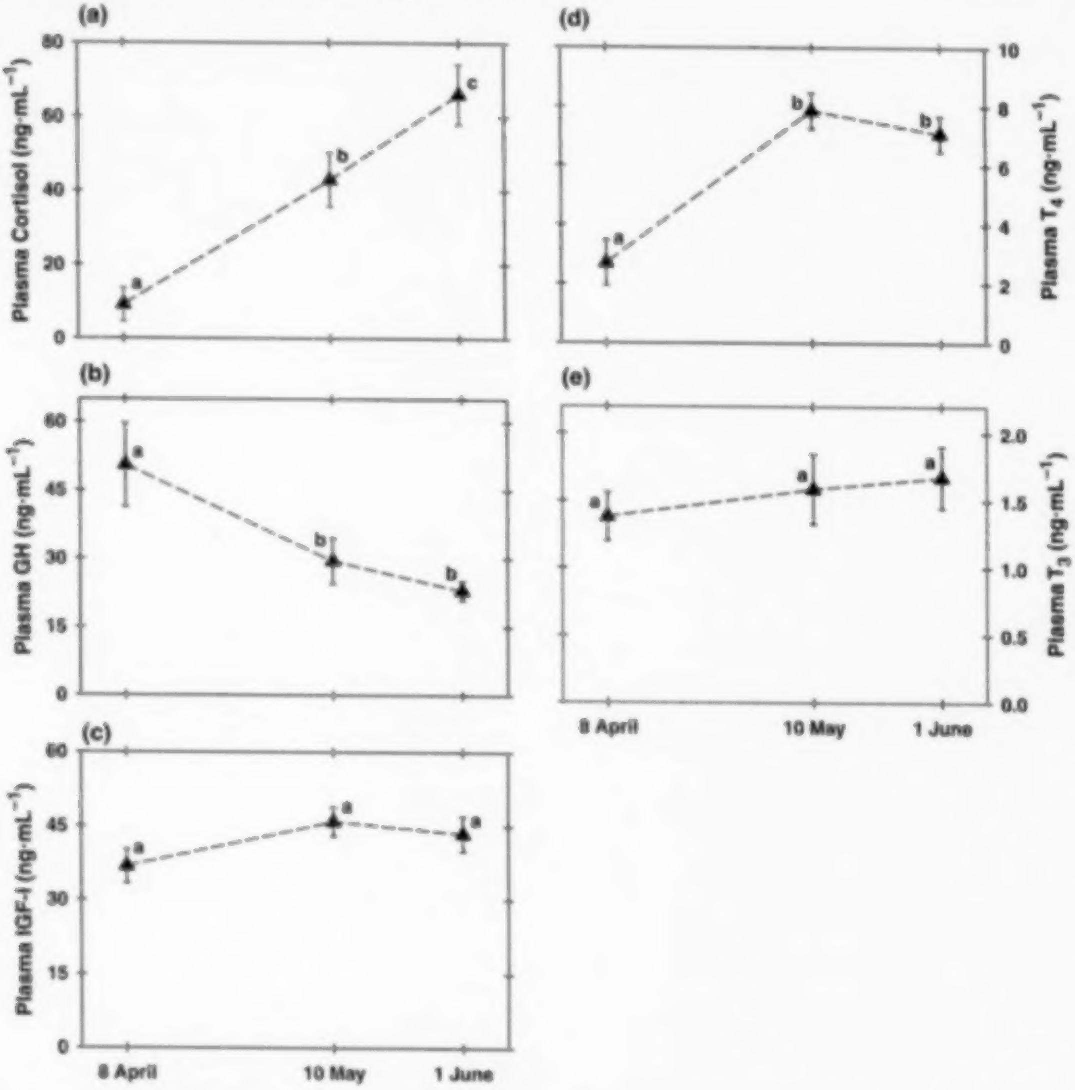


Results

Fish material

All fish sampled had a silvery body coloration, were immature, had empty stomachs, and displayed indices of previous exposure to marine parasites. Age of the fish ranged from 5 to 7 years, with mean age being 5.5 ± 0.1 years and with no significant differences in fish age between sampling dates. For fish sampled in FW, BM and FL were 314 ± 38 g and 33.3 ± 1.4 cm, respectively, in April, 350 ± 19 g and

Fig. 2. Plasma hormone levels of wild anadromous Arctic char (*Salvelinus alpinus*) sampled during ice-fishing on 8 April (n = 5) and 10 May (n = 7) and during downstream migration on 1 June (n = 7). (*a*) Cortisol; (*b*) growth hormone (GH); (*c*) insulin-like growth factor-I (IGF-I); (*d*) thyroxine (T_4); (*e*) 3,5,3′-triiodothyronine (T_3). Values are means ± SE of the mean. Different letters indicate a significant difference in this parameter between samplings (ANOVA, $p < 0.05$).



35.4 ± 0.9 cm in May, and 314 ± 19 g and 34.4 ± 0.6 cm in June. There were no significant differences in BM and FL between the sampling dates. Further, there were no significant differences in BM, FL, or Kf between downstream migrants sampled in FW and those sampled during the SWT. The Kf of fish sampled in FW is presented in Table 1.

Gill Na^+/K^+ ATPase activity and hyposmoregulatory capacity

Gill Na^+/K^+ ATPase activity was low in April and increased steadily and significantly (greater than fourfold) to June (Fig. 1). Plasma osmolality and chloride levels of descending migrants sampled in FW were 316 ± 2 mosmol·kg water^{-1} and 141 ± 1 mmol·L^{-1}, respectively. These levels were significantly higher in fish subjected to SWT, with plasma osmolality and chloride concentration being 345 ± 5 mosmol·kg water^{-1} (i.e., a 9% increase compared with FW) and 152 ± 3 mmol·L^{-1}, respectively (i.e., an 8% increase compared with FW) following 48 h of SW exposure. During the SWT, plasma osmolality tended to be highest at 6 h (353 ± 5 mosmol·kg water^{-1}) but with no significant differences between sampling times (3, 6, 24, and 48 h). A similar trend was evident for plasma chloride concentration, which was significantly higher at 3 h (being 164 ±

2 mmol·L^{-1}) than at 6, 24, and 48 h. There was no mortality during the SWT.

Plasma hormone levels

We present the plasma levels of cortisol, GH, IGF-I, T_4, and T_3 in Fig. 2. Plasma cortisol levels increased significantly from April to May and from May to June. Plasma GH levels decreased significantly from April to May, after which there was no significant change. Plasma T_4 levels increased significantly (approximately threefold) from April to May, after which there was no further change. There were no significant differences in plasma IGF-I and plasma T_3 levels between sampling dates.

Intermediary metabolism

Liver protein concentration was 155 ± 3 mg protein·g liver^{-1}, with no significant differences between sampling dates. Liver mass (wet mass) was 2.3 ± 0.3, 3.3 ± 0.2, and 2.9 ± 0.2 g for fish sampled in April, May, and June, respectively, with the liver mass of fish sampled in May being significantly higher than in fish sampled in April. The results obtained for other metabolic parameters are provided in Table 1. The Kf did not differ significantly between sampling dates. Plasma glucose concentration increased significantly from April to May, but no change was seen thereafter. There were no significant differences in plasma lactate concentration between sampling dates. Several parameters of liver intermediary metabolism showed temporal changes during the sampling period, with most of them taking place from April to May. In this period, HSI increased significantly (by almost 30%), concomitant with a significant reduction in liver glycogen stores (by 45%), after which there were no significant changes in these parameters. During the same period (April–May), activities of liver LDH (50%), PEPCK (160%), AlaAT (188%), AspAT (103%), and G6PDH (96%) increased significantly. No further changes in the activities of the above enzymes were evident from May to June. Liver PK activity showed a gradual, but significant increase by 125% over the whole sampling period from April to June. Liver HOAD activity did not change from April to May but increased significantly by 30% from May to June. Liver glucose concentration did not change significantly from April to May but decreased significantly by 63% from May to June.

Discussion

The present data on wild, anadromous Arctic char provide insights into how osmoregulatory, metabolic, and hormonal processes are integrated in wild fish in their natural environment. The exposure to marine parasites and the silvery body coloration in the FW Arctic char confirmed that all fish sampled in this study had an already established anadromous life history, implying that they had undergone their developmental parr–smolt transformation in a previous year. Consequently, the results in the present study demonstrate a resumption of smoltification processes including development of hyposmoregulatory capacity, increased intermediary metabolic capacities, and seaward migratory behaviour and that hormones such as cortisol, GH, and T_4 are involved in the integrated regulation of these processes.

Hyposmoregulatory capacity and role of hormones

In the present study, overwintering anadromous Arctic char displayed a fourfold seasonal increase in gill Na$^+$/K$^+$ ATPase activity, with the highest activity levels seen in descending migrants that also possessed a fully developed hyposmoregulatory capacity (cf. Hoar 1988; Nilssen et al. 1997). Similar results have also been observed in wild Arctic char that were captured on their annual return from SW and subjected to SWTs in the laboratory the following spring (Ø. Aas-Hansen, M.M. Vijayan, H.K. Johnsen, C. Cameron, and E.H. Jørgensen, unpublished data). These data suggest that repeat migrants of anadromous Arctic char lose their SW tolerance during overwintering in FW and redevelop this ability prior to successive SW migrations. Further support for this argument stems from the observation that hyposmoregulatory capacity was reduced in wild, anadromous Arctic char 3 weeks after their return from SW (Nilssen et al. 1997).

In the present study, the seasonal increase in gill Na$^+$/K$^+$ ATPase activity was paralleled by increased plasma cortisol levels, which is consistent with the well-established role of this hormone in stimulating gill Na$^+$/K$^+$ ATPase activity and hyposmoregulatory capacity during smoltification (Hoar 1988). The plasma levels of GH and IGF-I are often also reported to increase during salmonid smoltification (Björnsson 1997) and are known to stimulate gill chloride cell differentiation and Na$^+$/K$^+$ ATPase activity (McCormick 2001). This contrasts with the plasma profile of these hormones seen in repeat migrants of anadromous Arctic char in the present study, where increasing gill Na$^+$/K$^+$ ATPase activity was associated with unchanged plasma IGF-I levels and reduced plasma GH levels. The SW preadaptive stimulatory actions of GH and IGF-I have, however, been associated with reduced or unchanged plasma GH levels in smolting Atlantic salmon (McCormick and Björnsson 1994; Agustsson et al. 2001) and were suggested to be caused partly by increased GH-receptor binding in osmoregulatory tissues as seen during SW acclimation in rainbow trout (Sakamoto et al. 1990). It is possible, therefore, that a similar mechanism as well as GH-induced production of IGF-I in osmoregulatory tissues (Sakamoto et al. 1993) participate in the stimulation of hyposmoregulatory ability in repeat migrants of anadromous Arctic char. The pattern of vernally increased plasma T_4 levels and unchanged plasma T_3 levels in these char agree with the typically observed plasma profile seen during salmonid smoltification (Hoar 1988), with these hormones being considered permissive for hyposmoregulatory development (McCormick 2001) and with increased plasma T_4 levels being considered important for migratory behaviour (Iwata 1996).

Intermediary metabolism

The observation that all fish sampled in this study had empty stomachs confirms previous studies demonstrating that anadromous Arctic char experience prolonged fasting during overwintering in FW (e.g., Boivin and Power 1990). Prolonged fasting in fish is normally associated with a reduced HSI and liver metabolic capacity, including reduced plasma glucose levels (Foster and Moon 1991; Bastrop et al. 1992; Navarro and Gutierrez 1995). Although these characteristics seem consistent with the metabolic parameters of the fish sampled in April in the present study, the pronounced increases in HSI, liver enzyme activities, and plasma glucose levels seen from April to May are clearly not akin to fasting but rather indicate a reorganization and enhancement of liver metabolic processing capacity. This increased liver metabolic capacity may be a key aspect of the seasonal migratory strategy of this species to fuel energy-demanding processes (including the development of hyposmoregulatory ability) and to facilitate food acquisition and growth in the marine habitat.

Specifically, plasma glucose concentration, an important fuel for gill metabolism (Mommsen 1984), was higher in May and June relative to April and corresponded to increased gill Na^+/K^+ ATPase activity during the same period. The liver is the main site for de novo glucose production in fish (Moon and Foster 1995), and the increase in plasma glucose concentration also corresponded to elevated liver PEPCK activity (a key gluconeogenic enzyme) and aminotransferase activities (AlaAT and AspAT), indicating a concomitantly enhanced capacity for hepatic utilization of amino acids for oxidation and gluconeogenesis. Similar increases in liver gluconeogenic capacity from amino acids have also been reported during smoltification in coho salmon (*Oncorhynchus kisutch*) (Guillaume et al. 1984) and in migrating masu salmon smolts and postsmolts (*Oncorhynchus masou*) (Shikata 1998), prompting the latter author to suggest that this could be used as an indicator of successful SW preadaptation. The increased gluconeogenic capacity in May and June in the present study also coincided with an elevated LDH activity (an equilibrium enzyme catalysing pyruvate–lactate interconversion) and a tendency only for increased plasma lactate levels even in the char sampled during downstream migration. Lactate is considered a prime gluconeogenic substrate in the salmonid liver (Mommsen 1986; Moon and Foster 1995), and hepatic lactate utilization has been demonstrated to contribute to plasma lactate removal in rainbow trout (*Oncorhynchus mykiss*) (Milligan and Girard 1993). It appears likely, therefore, that the elevated LDH activity in the present study reflects an increased capacity for hepatic lactate utilization. Such a mechanism may be favourable during migration (cf. French et al. 1983; Milligan and Girard 1993) and is in line with the changes in liver PK activity (a key glycolytic enzyme) from April to June indicating a gradual increase in hepatic glycolytic capacity over the whole sampling period rather than an abrupt increase from April to May. The reduction in hepatic glycogen content observed from April to May corresponds to a characteristic drop in this parameter typically seen during salmonid smoltification (Hoar 1988), suggesting that glycogenolysis may have served a transient role in hepatic glucose mobilization pending the activation of gluconeogenic pathways. The lower liver glucose concentration seen in June compared with May may represent a temperature-induced increase in metabolic rate and suggests an actual increase in hepatic glucose efflux during this period of final hyposmoregulatory development.

The liver is quantitatively the principal site also for de novo synthesis of fatty acids in salmonids, with carbon residues released from amino acid deamination being preferred precursors (Tocher 2003). In the present study, G6PDH activity (an enzyme of the hexose monophosphate pathway that also provides reducing equivalents for lipogenesis) increased by almost 100% from April to May, indicating an increased capacity for hepatic fatty acid synthesis. Interestingly, this also coincided with increased HSI and aminotransferase activities, suggesting that amino acid derived lipid accumulation may have contributed to increased liver mass during this period. A higher lipogenic capacity may have adaptive significance for energy metabolism during the early marine phase (Stefansson et al. 2003) but may possibly also involve SW preadaptive differentiative processes (Sheridan 1989; Tocher et al. 2000). In agreement with this, the higher activity of the enzyme involved in beta-oxidation (HOAD) in June compared with April and May indicates an increased capacity for hepatic lipid catabolism at the time of SW descendence, which was also associated with a tendency for lower HSI in these migrants.

Metabolic roles of hormones

Cortisol is often implicated in the control of hepatic carbohydrate and protein metabolism in fish (Mommsen et al. 1999), and the proposed role of this hormone in increasing gluconeogenic and transaminase activities is supported by the present study. The drop in liver glycogen content seen from April to May might have been modulated by other factors possibly including T_4, as the plasma levels of this hormone also increased during this period and is reported to facilitate hepatic glycogenolysis and hyperglycaemia in fish (see Plisetskaya et al. 1983). A possible involvement of T_4 also in the increased G6PDH activities may be suggested based on their concomitantly elevated levels in May and June and a proposed metabolic state-dependent role of this hormone in stimulating hepatic lipogenesis through activation of the hexose monophosphate pathway (Hochachka 1962). T_4 is known to increase hepatic fatty acyl desaturation and elongation and subsequent mobilization and incorporation of polar lipids into biological membranes in other vertebrates (Hulbert 2000), and a SW preadaptive increase in these activities is, indeed, seen in smolting Atlantic salmon (Tocher et al. 2000). In accordance with this, T_4 is also found to increase hepatic lipid mobilization during smoltification, in part by interacting with cortisol, GH, and prolactin (Sheridan 1989). Similarly, cortisol administration has been reported to induce hepatic HOAD synthesis (and thus increasing the hepatic lipolytic capacity) in brook trout (Vijayan et al. 1991). Few studies have investigated the roles of GH or IGF-I on liver metabolism, but an in vitro lipolytic effect has been demonstrated in rainbow trout liver slices following salmon GH treatment (O'Connor et al. 1993). Fasting is, however, found to reduce hepatic GH sensitivity and subsequent IGF-I synthesis in fish (see Mommsen 1998; Perez-Sanchez and Le Bail 1999), but it is not known whether a similar mechanism also applies to the prolonged fasted Arctic char in the present study. In a study using genetically modified Arctic char, overexpression of a sockeye salmon (*Oncorhynchus nerka*) GH gene significantly reduced liver cell size as compared with untreated controls (Pitkanen et al. 2001). It is therefore possible that the increased HSI observed from April to May in the present study may have been facilitated by the concomitant drop in plasma GH levels.

The plasma hormone profiles observed in overwintering Arctic char in this study may also reflect metabolic roles of these hormones in extrahepatic tissues. During prolonged natural fasting in fish, energy demands are believed initially to be met by fat and later also by protein catabolism (Navarro and Gutierrez 1995). In this situation, elevated plasma GH levels are believed to stimulate peripheral lipid mobilization (Mommsen 1998; Perez-Sanchez and Le Bail 1999), whereas cortisol generally is known to exert a proteolytic effect in extrahepatic tissues (Mommsen et al. 1999). From April to May in the present study, reduced plasma GH levels and increased plasma cortisol levels coincided with the increased capacity of the liver for amino acid catabolism and indicate an increased mobilization of peripheral protein from May onwards. Cortisol and T_4 are, however, known also to facilitate lipid mobilization in fish (Plisetskaya et al. 1983; Mommsen et al. 1999), and the higher plasma levels of these hormones in May and June compared with April may thus have ensured continued stimulation of peripheral lipolysis. The increase in plasma T_4 levels observed in the present study does, however, contrast with the general view that thyroid hormones reflect the nutritional state of salmonids, with plasma T_4 and T_3 levels typically being depressed in response to prolonged fasting (Eales 1988). It has been demonstrated in Arctic char that increased plasma T_4 levels are associated with very high feed rations, whereas plasma T_3 levels increase with increasing ration and display a strong positive correlation with specific growth rate (Eales and Shostak 1985). Recently, it has been demonstrated that the main pathway whereby T_4 exerts its effects is via changes in membrane acyl composition, resulting in greater metabolic capacities and allowing higher growth rates but also resulting in higher food requirements (Hulbert and Else 2000; Hulbert 2000). Accordingly, in the present study, the consistently low plasma T_3 levels presumably reflect the natural fasting state experienced by overwintering anadromous Arctic char, whereas the increased plasma T_4 levels coincided with the increased liver metabolic capacities and may have enhanced the metabolic scope for growth.

In conclusion, the present data indicate that the migratory strategy of anadromous Arctic char involves a shift from an energy-conserving overwintering state to a state where the fish become osmotically and metabolically prepared for their annual feeding migration to SW. The hormones cortisol, GH, and T_4 may be directly involved in this metabolic shift but may simultaneously also stimulate redevelopment of hyposmoregulatory ability as part of a seasonal resmoltification process.

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The impact of a benthic filter feeder: limitations imposed by physical transport of algae to the benthos

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Abstract: We used an acoustic Doppler profiler to investigate the hydrodynamics of a nearshore site in western Lake Erie, and we incorporated the measured parameters in numerical simulations of phytoplankton consumption by benthic zebra mussels (*Dreissena polymorpha*) to examine the link between pelagic production and benthic filter feeders. Daily-averaged eddy diffusivities varied from 10^{-5} to 10^{-4} $m^2 \cdot s^{-1}$ at our site. Our simulations demonstrate that diffusivities of this order decrease near-bed algal biomass, while algal biomass in the pelagic remains relatively unaffected. Between 8% and 67% of the algal biomass in the water column could be consumed daily, depending on the shape and magnitude of the diffusivity profile. Correspondingly, in situ vertical biomass profiles showed a near-bed zone of algal depletion, but no impact was observed near the surface. The impact of the zebra mussel in nearshore regions is expected to be stronger than in deeper open water. The flow of algal biomass into the benthos was tightly coupled with turbulent mixing, suggesting that open water algal consumption by zebra mussels is small compared with previously published estimates that ignored vertical turbulent mixing processes.

Résumé : Un courantomètre acoustique à effet Doppler nous a permis d'étudier l'hydrodynamique d'un site près de la berge dans la région occidentale du lac Érié; nous avons incorporé les variables mesurées dans des simulations numériques de la consommation de phytoplancton par des moules zébrées (*Dreissena polymorpha*) afin d'examiner le lien qui existe entre la production pélagique et les organismes filtreurs benthiques. Les diffusivités turbulentes moyennes varient de 10^{-5} à 10^{-4} $m^2 \cdot s^{-1}$ à notre site d'étude. Nos simulations montrent que des diffusivités de cet ordre de grandeur diminuent la biomasse des algues près du coussin de moules, alors que la biomasse des algues dans la zone pélagique demeure relativement inchangée. Entre 8 % et 67 % de la biomasse d'algues présente dans la colonne d'eau peut être consommée à chaque jour, selon la forme et l'importance du profil de diffusivité. De la même façon, les profils verticaux de biomasse en place montrent l'existence d'une zone de densité réduite d'algues près du coussin de moules, mais aucun impact près de la surface. L'effet des moules zébrées dans les régions près des berges doit vraisemblablement être plus grand que dans les eaux ouvertes plus profondes. Le flux de la biomasse des algues vers le benthos est étroitement relié au brassage turbulent, ce qui laisse croire que la consommation d'algues d'eaux libres est faible par rapport aux estimations publiées qui ne tiennent pas compte des processus de brassage turbulent vertical.

[Traduit par la Rédaction]

Introduction

Despite the investment of substantial resources and effort to evaluate the impact of the zebra mussel (*Dreissena polymorpha*) on aquatic ecosystems, a number of issues remain largely unresolved. The zebra mussel was introduced into the Great Lakes in the late 1980s. Neither the zebra mussel's direct impact on Great Lakes phytoplankton and zooplankton community dynamics nor its indirect effect on fish community stability is well understood. As their benthic habitat and lack of mobility limit their access to food, zebra mussels depend on transport mechanisms such as settling (algal sinking), turbulent vertical transport, horizontal advection, and resuspension to bring food to them. Thus, any eval-

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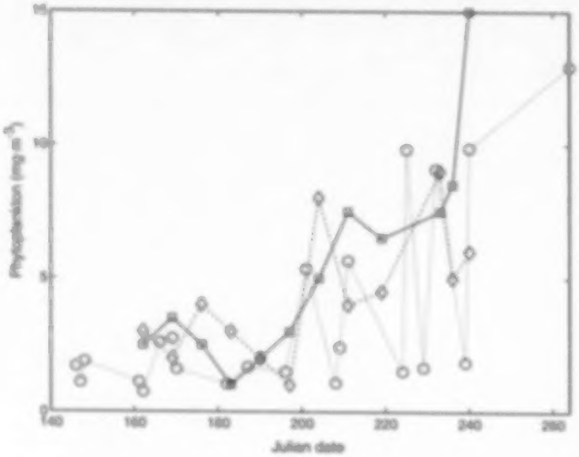
uation of the impact of zebra mussels on the phytoplankton community must be based on a fundamental understanding of both phytoplankton dynamics (changes in population size owing to mortality and reproduction) and physical dynamics (transport throughout the water column owing to turbulent mixing, advection, swimming, or settling) within the system. We use field measurements to investigate the mixing and parameterize a mathematical model of phytoplankton transport resulting from turbulent mixing as a contribution to the understanding of the dynamics of zebra mussel – algae interactions.

While the zebra mussel represents a new and insatiable consumer of algae, its filtering ability has not completely overwhelmed phytoplankton production, in part because algal consumption rates are limited by the rate of delivery of algae to the bottom. Using estimates of mussel population densities and filtering rates, other researchers have speculated that zebra mussels could strip the entire Lake Erie western basin of algae in less than 1 day, or even upwards of 18 times per day (MacIsaac et al. 1992; Bunt et al. 1993; Makarewicz et al. 1999). However, although phytoplankton abundances are now lower and cyanobacteria blooms less frequent than they were prior to 1988, the algal seasonal succession patterns have persisted (Wu and Culver 1991) (Fig. 1). These studies of zebra mussel feeding rates have been based on laboratory work using well-mixed tanks rather than realistic field conditions taking into account the algal delivery rates to the lake bottom and mussels' repeated filtering of the same water (Yu and Culver 1999). In addition, recent research indicates a strong correlation between mussel grazing and hydrodynamic forcing (MacIsaac et al. 1999).

Neglecting the effects of physical forcing in models of trophic dynamics of the Lake Erie ecosystem limits the models' appropriateness both as management tools and as a means to further our basic understanding of lake ecosystems. For example, western Lake Erie is commonly described as completely mixed, and estimates of zebra mussel grazing are based on this assumption (e.g., MacIsaac et al. 1992). Despite the lack of a seasonal thermocline, this assumption is unfounded. Further, marine ecologists have begun quantifying the relationship of fluid mechanics and benthic filter feeders. For example, Muschenheim (1987) studied the feeding ecology of a marine polychaete and concluded that most food is transported horizontally in the low-flow water near the bottom (concentration boundary layer). Vertically settling seston becomes potential food when it enters this boundary layer, but a strong concentration gradient develops with a localized depletion zone near the bed when the filter-feeding organisms remove seston faster than it is replaced. Other researchers have also found a decrease in phytoplankton concentration near marine benthic filter feeders in the field (Frechette and Bourget 1985*a*; Peterson and Black 1987; Peterson and Beal 1989) and in laboratory experiments (Wright et al. 1982; Wildish and Kristmanson 1984). Vertical feeding height (e.g., height above the bottom) attained by an organism (Jumars and Nowell 1984; Monismith et al. 1990) can thus determine the quantity and quality of its food supply and its rate of growth (Frechette and Bourget 1985*b*; Frechette et al. 1989).

Fig. 1. Seasonal patterns of algal biomass (wet weight from calculated volumes) before (1988, squares) and after (1989, diamonds; 1998, circles) zebra mussels (*Dreissena polymorpha*) became abundant in Lake Erie. Data from 1988 and 1989 (Wu and Culver 1991) are from weekly 3-m integrated water samples (cf. Wu and Culver (1991) for complete methodology). Data from 1998 are from the Lake Erie Plankton Abundance Survey (Ohio Division of Wildlife) and are averages of 20 weekly 3-m integrated water samples taken from 20 western basin stations.



These marine intertidal zones have long fetches and large tidal fluxes providing more constant and much larger turbulent energy inputs than those available in lakes such as Lake Erie. MacIsaac et al. (1992, 1999) found algal depletion in the concentration boundary layer (1.5 m thick) over zebra mussel beds in the western basin of Lake Erie, suggesting that algal delivery in large lakes may also be impacted by vertical mixing. Because mixing processes in lakes are less constant and weaker than their marine counterparts, we hypothesize that the availability of food for the benthic filter feeders, such as the zebra mussel, is even more limited by vertical mixing caused by differential flow or shear within the boundary layer than that demonstrated for marine systems.

Zebra mussels represent a large benthic biomass in western Lake Erie (150 g dry soft tissue·m^{-2} at our sampling site in 1995) and excrete large amounts of ammonia and phosphate (Arnott and Vanni 1996; James et al. 1997). At the same time, returning these nutrients to the euphotic zone also requires a mass transport mechanism. Therefore, understanding the role of nutrient and phytoplankton transport in the Lake Erie ecosystem requires identifying and quantifying the important physical, biological, and chemical processes controlling that transport.

We carried out a series of field experiments in the shallow western basin (mean depth = 7 m) of Lake Erie to evaluate shear velocity and estimate eddy diffusivity, measures of vertical mixing intensity. We then used these vertical mixing estimates in a series of simulations to determine the impact of the hydrodynamic forcing on the delivery of algae to the benthos. Finally, we tested these simulations by comparing the results with observed vertical algal distributions and estimating algal turnover rates corresponding to our simulations.

Materials and methods

Models

The abundance of algae in the concentration boundary layer depends on the algal settling rate, horizontal advection of phytoplankton, turbulent mixing above the mussel bed, the clearance rate of the zebra mussels, and external sources and sinks resulting from phytoplankton production and grazing by other organisms such as zooplankton (Cloern 1991; Lucas et al. 1999). The interaction of these physical and biological processes ultimately determines how much phytoplankton will be available to the zebra mussels at the bed. In this paper, we have neglected horizontal advection of phytoplankton to compare with earlier zebra mussel consumption estimates (MacIsaac et al. 1992, 1999; Bunt et al. 1993).

Finite mixing model

Under the above conditions, the effects of vertical mixing, settling, production, and zebra mussel consumption on the phytoplankton biomass can be modeled with a one-dimensional advection–diffusion equation (Cloern 1991):

$$(1)\qquad \frac{\partial F}{\partial t} - w_s\frac{\partial F}{\partial z} = \frac{\partial}{\partial z}\left(K\frac{\partial F}{\partial z}\right) + \mu F$$

where F is the concentration of the phytoplankton, t is time, w_s is the settling velocity of the phytoplankton (positive downward), z is the vertical coordinate, K is the vertical eddy diffusivity, and μ is the phytoplankton turnover rate. The boundary conditions in turn involve the total flux (vertical transport) of phytoplankton. At the water surface ($z = H$), there is no flux and hence no loss or gain of phytoplankton.

$$(2)\qquad -w_sF - K\frac{\partial F}{\partial z} = 0 \quad \text{at } z = H$$

At the bottom ($z = 0$), the phytoplankton flux equals the consumption rate of the zebra mussels (Frechette et al. 1989):

$$(3)\qquad -w_sF - K\frac{\partial F}{\partial z} = -\alpha F \quad \text{at } z = 0$$

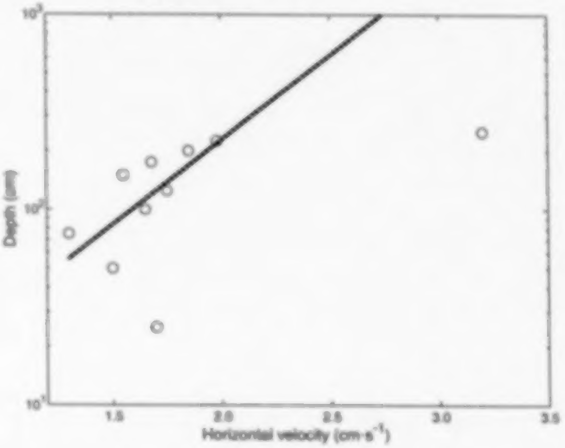
where α is the clearance rate of the mussels and αF (evaluated at the bottom) is the algal consumption rate. For initial conditions, we use a uniform algal distribution with depth.

Before solving the system defined by eqs. 1–3, we specify values for the parameters. We used a settling velocity, the rate of algal sinking, of 1 m·day^{-1} (Hutchinson 1967) and a zebra mussel clearance rate α = 25 m^3·m^{-2}·day^{-1} (see Discussion). The phytoplankton turnover rate μ was weighted according to a typical western basin light penetration curve, with a 10% light penetration point corresponding to 2 m beneath the surface. We estimated the eddy diffusivity assuming rough, turbulent flow, which can be described by a logarithmic velocity profile (Hinze 1975):

$$(4)\qquad u = \frac{u_*}{\kappa}\ln\left(\frac{z}{z_0}\right)$$

where $u_* = \sqrt{\tau_0/\rho}$ is the shear velocity, τ_0 is the shear stress on the bed, ρ is the water density, κ = 0.4 is the von Kármán constant, and z_0 is a length scale related to the bottom roughness. This assumption holds true above any bed with rough turbulent flow, as in Lake Erie (Chriss and Caldwell 1982; Bedford and Abdelrhman 1987). Estimates of u_* and z_0 can be obtained by measuring the velocity at a series of points above the bed. When the horizontal component of velocity is plotted against the natural logarithm of the distance above the bottom (eq. 4) (e.g., Fig. 2), u_* and z_0 can be computed from the slope and intercept, respectively. We assume that the turbulent diffusivity of phytoplankton is equal to that of momentum and that the mixing is due to the shear on the bed. Then the diffusivity has a parabolic distribution with depth (Fischer et al. 1979, p. 106):

Fig. 2. Regression fit of time-averaged horizontal velocities versus ln z used to estimate u_*, the shear velocity, and z_0, the roughness length, for 12 September 1995. The theoretical distribution of the logarithm of depth versus velocities above a bed is linear, necessitating the use of a linear fit in all profiles. This fit is for one 20-min period (r^2 = 0.75).



$$(5)\qquad K = \kappa u_* z\left(1 - \frac{z}{H}\right)$$

for which the depth-averaged diffusivity is

$$(6)\qquad K_{\text{avg}} = \frac{\kappa}{6}u_*H$$

Both the parabolic and uniform distributions of diffusivity are used in the simulations.

After the parameters were specified, the system (eqs. 1–3) was solved numerically, similar to the approach of Koseff et al. (1993), using the Crank–Nicolson method with central differences for the diffusion terms and an upwind difference for the sinking term. Following Koseff et al. (1993), a small, minimum eddy diffusivity was added to the profile in eq. 5; the minimum was typically 100 times smaller than the depth-averaged diffusivity. This minimum diffusivity prevents the generation of artifacts unrelated to the mixing profiles in the simulation results caused by an artificial barrier to mixing when the diffusivity approaches zero. For the range of parameters relevant for the western basin of Lake Erie, the simulation results are insensitive to the value of the minimum eddy diffusivity. We adopted a fully implicit formulation for the boundary conditions, since spurious oscillations develop when the Crank–Nicolson scheme is used (Douglas 1961). Typical values for the time step and grid spacing were 30 s and 0.035 m, respectively. A set of simulations was run to confirm that the results did not depend on these choices and that they matched analytical solutions for simple cases.

Well-mixed reactor model

The simulations of the effects of mixing and mussel grazing on algal biomass dynamics using eq. 1 are compared with two previous methods of estimating zebra mussel impact: the well-mixed reactor model and the no-refiltration model. The well-mixed reactor model assumes that the lake is well mixed, i.e., the algal concentration is uniform over the water column. Integrating eq. 1 over the depth yields an equation for the total phytoplankton biomass: $B = \int_0^H F\,dz$; then in the limit of large eddy diffusivity (and constant μ profile), one can show that (e.g., Koseff et al. 1993):

$$(7) \qquad \frac{dB}{dt} = B\left(\mu - \frac{\alpha}{H}\right)$$

The solution to eq. 7 is an exponential function in which the growth or decay rate depends on mussel consumption and phytoplankton production:

$$(8) \qquad B = B_0 e^{(\mu - \alpha/H)t}$$

where B_0 is the initial algal biomass.

No-refiltration model

The third and last model, which we call the no-refiltration model, corresponds to combining the pumping rate of mussels with areal estimates of zebra mussel abundance to estimate the amount of water filtered by mussels per square metre per day. It is then assumed that there is no refiltration and that algal concentrations remain constant in water being ingested by the mussels. These assumptions lead to a linear decline of algal biomass (milligrams per square metre) in the water column with time:

$$(9) \qquad B = B_0\left(1 - \frac{\alpha}{H}t\right)$$

In order for the assumptions of this model to hold true, the lake must remain completely static and algal sinking rates must equal the zebra mussel clearance rate α. Despite the unusual nature of these assumptions, this model has been used to obtain a theoretical maximum value for mussel impacts on Lake Erie (MacIsaac et al. 1992; Bunt et al. 1993). To include production, the biomass becomes $B = B_0[(1 + \alpha/\mu H)e^{\mu t} - \alpha/\mu H]$, which deviates from linear decay except for $\mu t << 1$. However, this modification is not examined further because eq. 9 is used for comparison with previous work, which did not include production terms.

Whether a water body with zebra mussels is well mixed depends on the speed of the various processes affecting the phytoplankton. For example, Koseff et al. (1993) defined several time scales: $T_{\mathrm{Graze}} = H/\alpha$ is the time for zebra mussels to filter the equivalent of the water column, $T_{\mathrm{Mix}} = H^2/K$ is the time for turbulence to mix the water column, $T_{\mathrm{Sink}} = H/w_s$ is the time for a particle to sink from the surface to the bottom, and $T_\mu = \mu^{-1}$ is the production time scale. An additional time scale is the duration of the simulation, which was held constant at 24 h. In general, the full model expressed by eqs. 1–3 accounts for all four processes or time scales, while the no-refiltration and well-mixed models do not account for mixing and sinking. (The no-refiltration model does account for sinking. In a way, the well-mixed reactor model also includes sinking, but in the limit of infinite K^* (high eddy diffusivity), the sinking is unimportant, as we show.)

Comparing systems with different forcing or biological parameters can be facilitated by forming ratios of the time scales. We define the three dimensionless ratios:

$$(10) \qquad K^* = \frac{K_{\mathrm{avg}}}{\alpha H} = \frac{T_{\mathrm{Graze}}}{T_{\mathrm{Mix}}}$$

$$(11) \qquad w_s^* = \frac{w_s}{\alpha} = \frac{T_{\mathrm{Graze}}}{T_{\mathrm{Sink}}}$$

$$(12) \qquad \mu^* = \frac{\mu H}{\alpha} = \frac{T_{\mathrm{Graze}}}{T_\mu}$$

Then, for example, a large value of K^* implies that turbulence mixes the water column much faster than the zebra mussels can filter the equivalent of the depth. In these terms, one would expect the agreement between the predictions of the full model and the well-mixed model to improve when K^* becomes large. Furthermore, the nondimensional sinking rate w_s^* allows comparison between the mussel clearance rate and the sinking rates of the algae. We have varied these nondimensional parameters and presented the effect on algal clearance. The well-mixed reactor is initially set at $\mu^* = 0$ and $K^* \to \infty$ and the no-refiltration model has $K^* = \mu^* = 0$ and $w_s^* = 1$.

Field methods

Field experiments were conducted in the western basin of Lake Erie (United States) offshore of Gibraltar Island

Fig. 3. Location of the sampling site between Gibraltar Island and Middle Bass Island in the western basin of Lake Erie. The inset map shows the location within the Great Lakes.

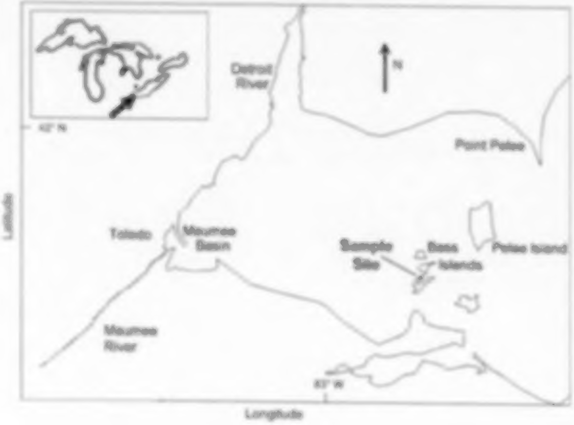


Table 1. Physical parameter variation at our sample site.

Date	Water velocity ($cm{\cdot}s^{-1}$)	Wind velocity ($m{\cdot}s^{-1}$)	z_0 (cm)	u_* ($cm{\cdot}s^{-1}$)	K_{avg} ($\times10^{-4}\ m^2{\cdot}s^{-1}$)
5 June	0.93	2–5 east	22	1.09±0.13	0.36
6 June	1.18	1–2 northeast	13	1.0±0.04	0.33
8 June	1.25	1–2 northeast	7.3	1.32±0.22	0.44
11 June	1.50	2–5 northwest	19	0.80±0.12	0.26
12 June	1.62	2–5 north	11	0.77±0.13	0.25
13 June	1.16	5–10 west	11.2	0.95±0.29	0.31
20 June	1.89	1–2 north	17	0.63±0.17	0.21
22 June	1.57	2–5 northeast	8.5	1.66±0.69	0.55
24 Aug.	4.63	2–5 north	12	0.19±0.12	0.06
25 Aug.	3.08	2–5 northwest	20.4	0.19±0.07	0.06
29 Aug.	1.84	2–5 northeast	13	0.32±0.10	0.10
12 Sept.	2.92	2–5 southwest	12	0.37±0.05	0.12
13 Sept.	4.63	5–10 south	18	0.13±0.12	0.04

Note: Mean (24-h) horizontal water velocity at 1 m was measured using the acoustic Doppler profiler, and mean wind velocity and direction were obtained from the Great Lakes Forecasting System (Kelly et al. 1998). The shear velocity u_* and the roughness length z_0 were calculated by regression using eq. 4 with 20-min time intervals and averaging of the resultant shear velocities over 24 h for each date. Only profiles with $r^2 > 0.5$ were used to develop a daily average, and only those dates with more than 50% of their profile regressions with $r^2 > 0.5$ are included above. The mean diffusivity K_{avg} was calculated from the shear velocity using eq. 5 for the 5-m water column. The distribution of the shear velocities was examined with histograms and found to be normally distributed; confidence intervals were generated with normally distributed standard error in u_* above.

(41°40′N, 82°50′W) (Fig. 3). We sampled the bottom 3 m of the 5-m column of water (ranging from 4 to 6 m owing to seiche activities), approximately 50 m from shore, from June until September 1995. The bed consisted of 5- to 10-cm-diameter cobble, with zebra mussels in clumps ranging from 5 to 20 cm in diameter. The zebra mussel dominated the benthic community with between 10 000 and 20 000 $mussels{\cdot}m^{-2}$ and up to 150 g mussel dry soft $tissue{\cdot}m^{-2}$ (Pontius and Culver 2001).

We measured water velocity measurements using a Sontek 3-MHz acoustic Doppler profiler, which was deployed on a steel tripod facing downward, recording the data via a 60-m cable to a shore-based computer. Using the acoustic Doppler profiler, water velocities were measured in 25-cm strata (bins) from the bottom to 3 m above the bottom, with the computer recording time-averaged vertical profiles of the three-dimensional velocity fields every 20 min. We generated a power spectrum (Welch method) from a time series of horizontal velocities from 1 m above the bottom to determine whether the current-forcing processes present at this site were characteristic of the western basin as a whole. We sampled continuously throughout the summer, interrupted only by two electronic failures resulting from lightning strikes on 24 June and 25 August. The horizontal components of velocity were used to estimate u_* and z_0 and, in turn, the eddy diffusivity using eqs. 4–6. Profiles with good fits (linear regression, $p < 0.05$) were included in a 24-h average diffusivity (K_{avg} obtained from eq. 6 with 24-h-averaged u_* values). Wind speeds corresponding to the sample dates were obtained at 6-h intervals from the Great Lakes Forecasting System (Kelly et al. 1998) to compare with measured mixing parameters. Vertical temperature and oxygen profiles of the whole water column were taken daily (Yellow Springs Instruments dissolved oxygen probe, Yellow Springs, Ohio) to test for significant stratification during the sampling periods.

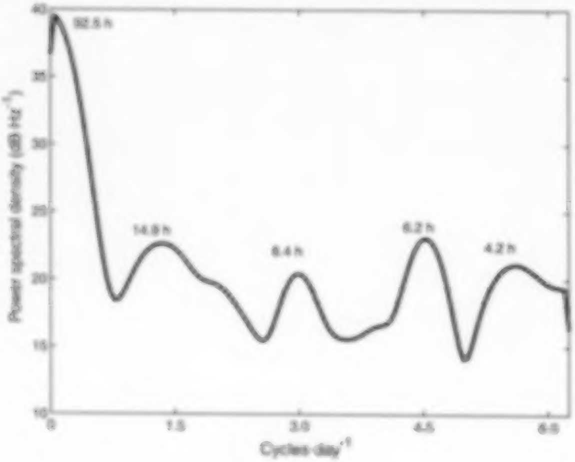
We measured vertical phytoplankton abundance distribution three times per week using two shore-based 10-channel peristaltic pumps. Water was sampled from an apparatus deployed on the acoustic Doppler profiler tripod at fixed depths of 6, 12, 24, 36, 49, 61, 98, 148, 200, and 250 cm above the bed. Tubes from the sampler passed to shore inside an opaque plastic pipe. The tubes were each flushed before sampling by passing twice the tube volume through the tubes prior to collecting samples at only 30 $mL{\cdot}min^{-1}$. The samples thus represent well the stratum from which each was taken and the 3-L samples used for analysis were integrated over time (~100 min). Phytoplankton samples were preserved using Lugol's fixative and were enumerated using an inverted microscope to produce phytoplankton biomass (milligrams wet mass per litre based on calculated cell biovolumes determined from linear cell dimensions) profiles versus depth for each sample date.

Results

Field results

The temperature varied less than 0.1 °C from the surface to the bed during the sampling period. Time-averaged mean horizontal currents ranged from 0.93 to 4.63 $cm{\cdot}s^{-1}$, while wind velocities from the same periods ranged from 1 to 15 $m{\cdot}s^{-1}$ (Table 1). Although water velocity changes did not appear to correspond to wind speed, the stronger currents tended to coincide with winds from the north. The power spectrum of current velocities 1 m above the bottom was calculated from 10 June 1995 until the first lightning strike on 23 June (Fig. 4) and shows large peaks at 92.5 and 14.9 h, with smaller peaks at 8.4, 6.2, and 4.2 h, with no diel (24-h) signal evident. Using the fit of time-averaged vertical profiles of horizontal velocity versus ln z (which have a slope of $u_*\kappa^{-1}$ and intercept the z axis at $z = z_0$), we calculated the shear velocities u_* and the roughness lengths z_0 for each

Fig. 4. Power spectrum of horizontal velocities at our sample site at 1 m above the bottom, June 1995. From left to right, peaks correspond to the storm cycle, first-mode seiche, second-mode seiche, third-mode seiche, and a combination of the fourth mode and cross-basin seiches.



date. For example, on 12 September, $u_* = 0.37$ cm·s^{-1} and $z_0 = 12$ cm (Fig. 2). The z_0 varied from 7 cm to as great as 20 cm, whereas individual zebra mussels on the bottom varied in size from 0.1 to 3.0 cm. The u_* values and the corresponding diffusivities depended primarily on wind direction (Table 1). The roughness Reynolds number u_*k_s/ν, where $k_s = z_0/30$ is the equivalent sand grain roughness of the bed and ν is the kinematic viscosity, always exceeded 70, the value required for eq. 4 to be valid (Hinze 1975). The lack of data in the two periods between 22 June and 24 August and again between 24 August and 11 September reflects the periods required for repairs after direct lightning strikes caused electronic failures in the acoustic Doppler profiler. We then estimated the depth-averaged eddy diffusivity using eq. 6. For example, evaluating a daily average K for 12 September 1995 gave $K_{avg} = 1.2 \times 10^{-5}$ m^2·s^{-1}. We thus evaluated K_{avg} for each date (Table 1), dropping those dates having weak fits ($p > 0.05$).

Algal biomass varied widely with depth in June and July 1995 (Julian days 92–152; Fig. 5, solid line), with values from samples near the benthos (*i.e.*, zebra mussels) being very low, while farther away, biomass increased exponentially above the bed (nonlinear regression, $p < 0.01$). During August and September, a different trend was apparent, although the variance was large in these samples (Julian days 174–200; Fig. 5, dashed line). Examination of the taxonomic composition in the algae samples indicated that phytoplankton biomass near the bottom was dominated by the cyanobacterium *Microcystis* (e.g., 81% of the algal biovolume in the August 98 cm sample), a vertically migrating (buoyant) species often rejected by zebra mussels (L. Babcock-Jackson, Chemical Abstracts Service, P.O. Box 3012, Columbus, OH 43210, USA, personal communication).

Fig. 5. Algal biomass (wet mass from calculated volumes) distributions for early and late summer versus depth above bed at a single site in western Lake Erie. The solid line indicates profiles before 1 August 1995 (mean of five profiles) and the dashed line is after 21 August 1995 until 15 September 1995 (mean of four profiles). The horizontal bars indicate standard deviation around the mean profile.

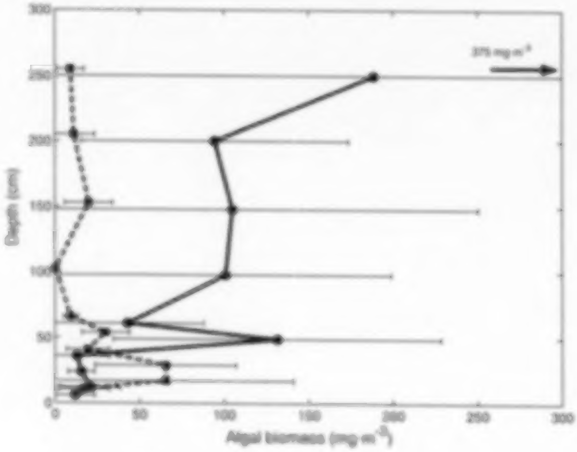
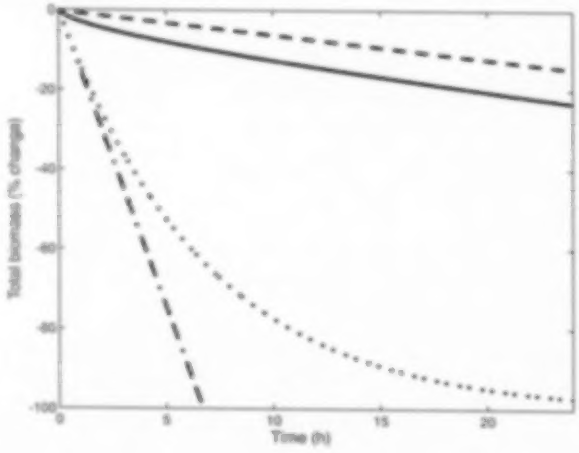


Fig. 6. Changes in total algal abundance computed from eq. 1 with parabolic eddy diffusivity distribution (solid line), eq. 1 with constant eddy diffusivity distribution (dashed line), well-mixed reactor model (dotted line), and no-refiltration model (dot-dashed line).



Simulation results

Simulations solving eq. 1 indicated less algal consumption than predicted by the well-mixed and no-refiltration approaches (Fig. 6). Twenty-four-hour consumption estimates are 15% and 20% for solutions to eq. 1 (parabolic and uniform distribution of K, respectively) compared with 97% at 24 h for the well-mixed model and 100% at 6.8 h for the no-refiltration model. Vertical biomass distribution after 24 h varies owing to both the magnitude and shape of the diffusivity distribution with depth (Fig. 7). The parabolic shape induced less algal clearance than did the uniform distribution with the same depth-averaged diffusivity. Lower values of diffusivity resulted in declining algae near the sur-

Fig. 7. Simulated biomass profiles after 24 h versus height above bed (*z*): solid line, parabolic eddy diffusivity with a mean of 10^{-4} m^2·s^{-1}; dot-dot-dashed line, uniform eddy diffusivity of 10^{-4} m^2·s^{-1}; dotted line, parabolic eddy diffusivity with a mean of 10^{-5} m^2·s^{-1}; dot-dashed line, uniform eddy diffusivity of 10^{-5} m^2·s^{-1}.

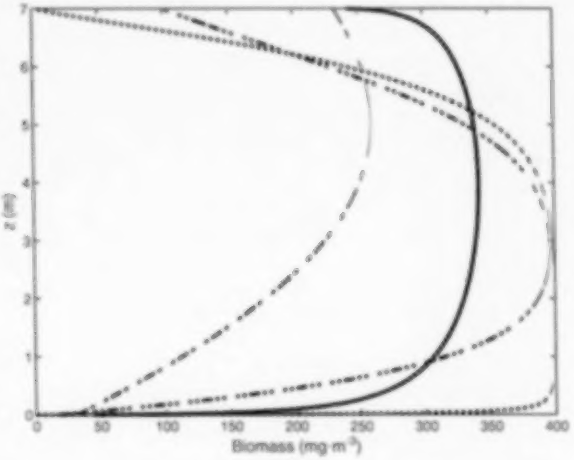
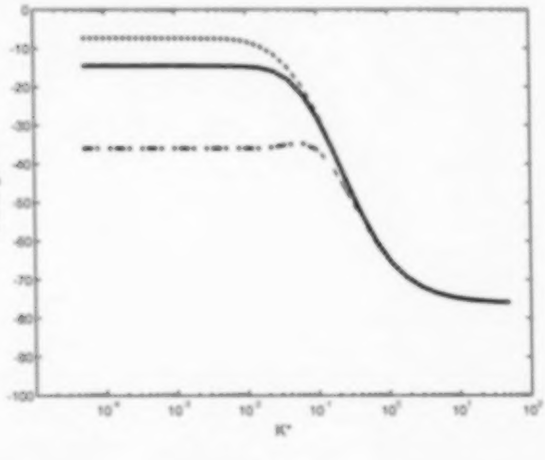


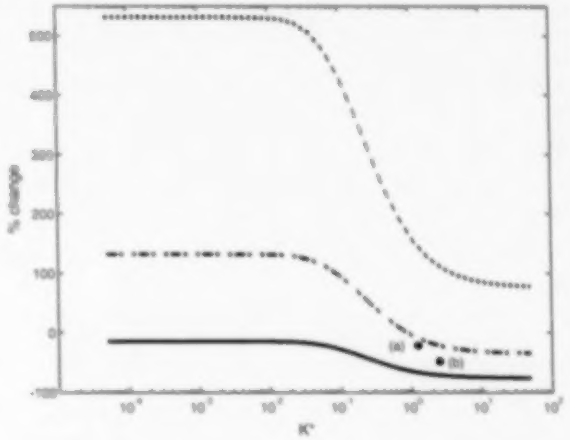
Fig. 8. Effect of mixing, expressed as a nondimensional diffusion coefficient (K^*), on the predicted percent change in total algal abundance over 24 h for three values of the nondimensional sinking rate (w_s^*): dotted line, $w_s^* = 0.02$; solid line, $w_s^* = 0.04$; dot-dash-dotted line, $w_s^* = 0.1$. The base values of α and *H* (zebra mussel clearance rate and water depth, respectively) were used and *K* was varied between 10^{-7} and 10^{-1} m^2·s^{-1}.



face and very strong gradient of algal concentration near the bed.

Increases in the nondimensional sinking rate w_s^* result in increased depletion, except at high values of K^* (Fig. 8). Inclusion of phytoplankton production results in increased algal abundance relative to estimates without production (Fig. 9). High production has the greatest impact at low values of K^*, while there is increased clearance at all levels of production at high K^*.

Fig. 9. Effect of mixing and production, expressed in nondimensional terms, on the predicted percent change in total algal abundance over 24 h with varying nondimensional algal production (μ*): solid line, μ* = 0; dot-dash-dotted line, μ* = 0.28; dotted line, μ* = 0.56. Markers indicate values calculated from Cloern (1991) (*a*) and Ackerman (2001) (*b*). The base values of α, w_s, and *H* (zebra mussel clearance rate, algal sinking rate, and water depth) were used and *K* was varied between 10^{-7} and 10^{-1} m^2·s^{-1}.



Discussion

Comparison with previous field measurements

These Lake Erie results are consistent with those from previous physical studies. We found that the power spectrum of horizontal velocities (at 1 m above bed) for the period of 10 June 1995 to 23 June 1995 corresponded to those found by Hamblin (1987) for the Lake Erie weather cycle (96 h), the first-mode seiches (14 h), the second-mode seiches (9 h), the third-mode seiches (6 h), and the cross-basin seiche (3 h). The second-mode seiches (9 h) are comparatively weak, as the sample site was very close to the second-mode node. A diel (24-h) signal was absent because the sampling captured only the influence of bed shear and could not detect convective events or diel thermocline formation because these do not influence horizontal currents. With this one exception, our results from a 5-m-deep near shore site have water currents characteristic of both the western basin and shallow regions of the lake as a whole. Thus, they can be used to study the effects of shear on grazing by zebra mussels.

Royer et al. (1987) found current velocities in the lake to vary from 1 to 10 cm·s^{-1} using several measuring methods, while Bedford and Abdelrhman (1987) predicted velocities of 1–5 cm·s^{-1} during calm weather and 10–20 cm·s^{-1} during storm events, both comparing favorably with our results. Shear velocities were slightly less than expected for our exposed site, varying from 0.13 to 1.66 cm·s^{-1}, almost one order of magnitude less than those predicted by Bedford and Abdelrhman (1987). However, the estimates of z_0 were higher than they predicted, perhaps because of the zebra mussel covered cobbles. Although the cobbles were on the order of 8–10 cm, the mussel druses (large clumps of zebra mussels) increased the size of the roughness elements to 20–30 cm or greater.

The magnitude of eddy diffusivities varied from 10^{-5} to 10^{-4} $m^2 \cdot s^{-1}$, with the largest values corresponding roughly to wind direction owing to the location of the sample site on the north side of an island. These values are consistently two orders of magnitude below the high diffusivities generated by tidal flows in shallow marine systems (on the order of 10^{-2} $m^2 \cdot s^{-1}$). Storm events in Lake Erie's much deeper central basin have been found to support diffusivities as high as 10^{-2} $m^2 \cdot s^{-1}$ (McCune 1998). That we did not obtain values this high in western Lake Erie was due, at least in part, to daily averaging of current speeds that, while more representative of conditions on the lake, could not capture short-term, high-intensity events such as severe storms. Storm events may be ecologically less important than less intense mixing events, since they can overwhelm the mussel filtering capacity; a large suspended sediment load causes the mussels to stop filtering (Ackerman 1999).

Mussel impacts on algae

We found a strong zone of depletion in algal biomass from samples near the benthos in early and midsummer. The mussels were able to consume much of the algae in the near-bed region, causing a zone of depletion similar to that found by MacIsaac et al. (1999). However, the algal abundance and composition of the upper water column remained similar to those found prior to the introduction of the zebra mussel (Wu and Culver 1991). In late August and into September, there was increased biomass (dominated by the cyanobacterium *Microcystis*) near the benthos, as opposed to a zone of depletion.

The trend in algal vertical distribution observed in August and September 1995, an increase in algal biomass above the mussels, may be explained by the resurgence of the toxic cyanobacterium *Microcystis*. A large bloom of *Microcystis* first appeared in August 1995 (Culver et al. 1999). Various researchers (Heath et al. 1995; Holland et al. 1995; Arnott and Vanni 1996) have attributed such blooms, including a subsequent bloom in 1998, to the excretion of nitrogen and phosphorus by zebra mussels. However, while *Microcystis* is therefore an integral part of the zebra mussel problem, it has two qualities that cause it to interfere with estimates of the impacts of the zebra mussels on the phytoplankton community: first, it migrates vertically to obtain the best light and nutrient regimens, and second, large colonies are rejected by zebra mussels and may reenter the water column (Vanderploeg et al. 2001). Thus the phytoplankton maximum observed herein may be then a product of both the alga's migration ($w_s \neq 1$) and the zebra mussels' preference not to consume it.

While we observed little evidence of algal depletion away from the benthos, others have estimated much greater consumption of algae from the whole water column by the zebra mussels based on filtering estimates (MacIsaac et al. 1992, 1999; Bunt et al. 1993) and bioenergetic models (Madenjian 1995). Their estimates of clearance rates (cubic metres per square metre per day) indicate that a volume equivalent to that of the entire water column can theoretically be filtered completely once per day by small-bodied individuals (Bunt et al. 1993) and between 3.5 and 18.8 times per day by the whole community (MacIsaac et al. 1992). However, it has been unclear how close to the theoretical maximum filtering (pumping) rate the mussel community actually achieves and what fraction of the algae in the water column is consumed per day. Yu and Culver (1999) found that refiltration of water previously cleared of algae can significantly decrease the mussels' impact on the algal population, while Hogan and Mills (1997) correspondingly observed decreased clearance rates as algal concentrations decreased.

Effects of finite mixing

We compared the algal consumption predicted by the no-refiltration model of MacIsaac et al. (1992) and others, the well-mixed reactor model, and the finite-mixing model. The solution of eq. 1 used $\mu = 0$ as in the two simpler models, an initially uniform algal concentration, and constant and parabolic eddy diffusivity distributions with a depth-averaged diffusivity of 10^{-4} $m^2 \cdot s^{-1}$, which is an upper bound on the average diffusivities measured in the field. The western basin of Lake Erie is not well mixed, so when the vertical mixing is finite, the predicted algal clearance determined by eq. 1 is much less than that from either the no-refiltration or the well-mixed model. Only 2% of the algae is removed from the lake (assuming a parabolic diffusivity distribution) after 6.8 h, when the no-refiltration model predicts that the algae have been completely filtered from the lake. After a day, the well-mixed model predicts that 97% of the algae have been consumed, while the solution of eq. 1 gives only 15% and 20% of the algae removed per day for depth-averaged eddy diffusivities of 10^{-5} and 10^{-4} $m^2 \cdot s^{-1}$. The values from the model with no-refiltration are similar to the 26 ± 10% predicted by Madenjian's (1995) bioenergetic approach. However, shallow waters will be affected more severely by the mussels' filtering ability. Reducing the water column height from 7 to 2 m causes the consumption results of eq. 1 to jump to 47% and 80% for $K = 10^{-5}$ and 10^{-4} $m^2 \cdot s^{-1}$, respectively. This result is consistent with other nearshore field research including changes in nearshore algal communities (Hatchery Bay, Lake Erie; Holland et al. 1995) and benthic invertebrates (Saginaw Bay, Lake Michigan; Nalepa et al. 2003).

The vertical distribution of the eddy diffusivity strongly affects the changes in the algal profiles over 24 h and hence the estimates of grazing rates by zebra mussels. The shape of the eddy diffusivity profile is important because the lower diffusivity near the bottom in the parabolic profile reduces algal transport to the benthos. When the eddy diffusivity is uniform over the depth, the consumption increases; clearance rates for the uniform diffusivity distribution after 24 h are 49% and 23% for eddy diffusivities of 10^{-4} and 10^{-5} $m^2 \cdot s^{-1}$, respectively. In addition, lower diffusivities near the bottom lead to very thin layers of algal depletion, and near the water surface, the mixing is too weak to counteract sinking.

Effect of time scales

Using the three dimensionless parameters, we systematically examined the effects of diffusivity, sinking rate, and algal reproduction rate. As the eddy diffusivity increases, the consumption rate increases for all values of sinking rate. At low K^*, sinking controls consumption, while at higher K^*, mixing controls consumption. In particular, for $K^* > 0.3$, the sinking rate is unimportant. When $K^* > 10$, the consumption no longer depends on K, and if a uniform eddy diffusivity profile were used, the clearance would be $97\%\cdot\text{day}^{-1}$ as in the well-mixed model. These results allow "well mixed" to be defined quantitatively. For example, for the western basin of Lake Erie to be well mixed, the eddy diffusivity would have to exceed $2 \times 10^{-2}\ \text{m}^2\cdot\text{s}^{-1}$, which is more than 100 times higher than our measured values. In addition, for the same K and α, a reduced depth increases K^*, resulting in greater algal clearance.

However, these estimates and our previous comparison with other works lack a fundamental part of the algal transport process and consumption dynamic: algal production. When we include productivity, we can compare our work with previous effort in other systems and even calculate values for K^* and μ^* from their data. Cloern (1991) first used a similar model in a marine tidal system with parabolic diffusivity profiles ($K = 5 \times 10^{-5}\ \text{m}^2\cdot\text{s}^{-1}$ and $K^* = 0.625$) and found algal removal rates of $6.3\%\cdot\text{day}^{-1}$. Increasing mixing by one order of magnitude increased consumption to $33\%\cdot\text{day}^{-1}$. His results include an algal source term, resulting in decreased clearing at low K^*. Ackerman et al. (2001) investigated the impacts of grazing across a single dreissenid mussel bed in western Lake Erie, estimating clearance from the beginning to the end of the bed in addition to physical parameters. At $K^* = 1.25$, they found 40% algal clearance across the bed, although without a source term. We find that our estimates of algal consumption are similar but diverge with increasing production. Higher production values and decreased mixing (K^*) can result in rapid increases in algal population, especially as the higher productivity regions (near the surface) are separated in space from mussel consumption. With high μ ($\mu^* = 0.56$) and high K^*, the increase becomes less profound, as the mussels have access to more of the water through increased mixing. Low productivity, $\mu^* = 0$, results in increasing depletion of the water column with that depletion strongly linked to mixing, K^*. Less extreme values in productivity, $\mu^* = 0.28$ (equivalent to one algal replication per day), result in modest increases or decreases, depending on the intensity of the mixing. This result appears most reasonable, given the lack of evidence for changes in seasonal algal abundance patterns in the lake since dreissenids became established (Wu and Culver 1991).

In summary, a series of field experiments in a nearshore region of Lake Erie produced diffusivity estimates that did not exceed $10^{-4}\ \text{m}^2\cdot\text{s}^{-1}$ (averaged over 24-h periods) despite small storm events. Because of the relatively weak mixing, the strong dependence of benthic feeding rates on hydrodynamic processes that has been consistently observed in the marine literature is even more important in large lakes. Modeled algal depletion was strongly linked to the magnitude and shape of the diffusivity profile, indicating both that the no-refiltration and well-mixed reactor models for estimating algal consumption grossly overestimate zebra mussel consumption and that there is a strong need for research into the shape of the diffusivity profiles in large lakes. Vertical algal profiles taken during the sampling verify that algal depletion is strongly influenced by hydrodynamic forcing. Finally, future efforts to estimate the impact of the benthos on the planktonic algal community must be linked to estimates of algal productivity and hydrodynamic forcing.

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A new likelihood for simultaneously estimating von Bertalanffy growth parameters, gear selectivity, and natural and fishing mortality

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Abstract: Gear selectivity and the cumulative effects of size-selective fishing produce bias in the length-at-age samples used to estimate the von Bertalanffy growth parameters. In fished populations, fast-growing young fish and slow-growing old fish are overrepresented in size–age samples. To account for such effects, we treated size-at-age observations as multinomial samples, with expected catches in each size–age category dependent on growth parameters, growth variation, size selectivity, abundance at age, and the history of exploitation. Using simulated data sets, estimated growth parameters using the multinomial likelihood were unbiased when fishing mortality was not too high and the shape of the vulnerability function was correct. In contrast, estimated growth parameters using a least squares approach overestimated the metabolic growth coefficient (K) and underestimated mean asymptotic length (L_∞). Models that do not explicitly account for the effects of fishing and size selectivity underestimated L_∞ and overestimated K. We estimate growth parameters for northern pikeminnow (*Ptychocheilus oregonensis*) as an example of the method and document a stunted "pigmy" population with an L_∞ of 175-mm fork length, attributing its small size to effects of high density and (or) a short growing season.

Résumé : La sélectivité des engins de pêche et les effets cumulatifs de la pêche sélective en fonction de la taille faussent les échantillons de longueurs en fonction de l'âge utilisés pour estimer les paramètres de croissance de von Bertalanffy. Dans les populations soumises à la pêche, les jeunes poissons à croissance rapide et les poissons âgés à croissance lente sont surreprésentés dans les échantillons de longueurs en fonction de l'âge. Pour tenir compte de ces effets, nous avons traité les observations de longueurs en fonction de la taille comme des échantillons multinomiaux, avec des captures attendues dans chaque catégorie de taille en fonction de l'âge dépendantes des paramètres de croissance, de la variation de la croissance, de la sélectivité en fonction de la taille, de l'abondance en fonction de l'âge et de l'exploitation faite au cours des ans. À l'aide de séries de données simulées, nous montrons que les paramètres de croissance estimés d'après la vraisemblance multinomiale ne sont pas faussés quand la mortalité due à la pêche n'est pas trop importante et lorsque la forme de la fonction de vulnérabilité est exacte. En revanche, le calcul des paramètres de croissance estimés par la méthode des moindres carrés surestime le coefficient de croissance métabolique (K) et sous-estime la longueur moyenne à l'asymptote (L_∞). Les modèles qui ne tiennent pas compte de façon explicite des effets de la pêche et de la sélectivité en fonction de la taille sous-estiment L_∞ et surestiment K. À titre d'exemple de notre méthodologie, nous faisons l'estimation des paramètres de croissance du méné-brocheton du nord, *Ptychocheilus oregonensis*, et nous présentons une population « pygmée » à croissance ralentie qui possède un L_∞ de 175 mm de longueur à la fourche, un effet que nous attribuons à la forte densité et (ou) à la courte saison de croissance.

[Traduit par la Rédaction]

Introduction

Estimating parameters for the von Bertalanffy growth model assumes that a representative sample of lengths from each age-class has been collected and then fitting the growth function either to length–age data directly or to data on changes in length between the time of marking and time of recapture. This assumption fails because the sampling process is size selective and because of the cumulative effects of fishing on size-at-age. Growth rates vary among individuals (Sainsbury 1980; Burr and Doksum 1988) and intensive size-selective harvesting removes faster growing individuals. The result is that length–age samples are biased for larger fast-growing young individuals and biased for older slow-growing individuals that avoided capture (Lee 1912; Ricker 1969; Sinclair et al. 2002*a*). In our experience, almost all length–age data sets collected for growth analysis involve size-selective capture methods that favor fast-growing individuals. In addition, many come from populations that have historically been exposed to harvest so as to produce cumu-

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lative effects on size structure at age (Hansen and Chouinard 1992; Kristiansen and Svasand 1998). In both cases, the result is a downward bias in estimation of mean asymptotic length (L_∞), upward bias of the metabolic growth parameter (K), and upward bias in the apparent size at hatching (t_0).

Biased estimates of growth parameters will distort bias mortality rate estimates (where length–age keys are used to convert length to age), biological reference points for management such as yield per recruit (Ricker 1969; Parma and Deriso 1990), and management actions (quotas, for example). Changes in size selectivity over the course of a fishery complicate matters further, where size selectivity changes may be interpreted as changes in growth rates. Sinclair et al. (2002*b*) documented changes in size selectivity for the Atlantic cod (*Gadus morhua*) fishery in the southern Gulf of St. Lawrence, and Sinclair et al. (2002*a*) noted that historical effects of temperature on measured annual growth increment and density-dependent increases in growth rate were relatively small in comparison with the effect of size-selective mortality caused by the fishery.

In some cases, researchers wish to solely estimate growth parameters describing the current population available to harvest with the understanding that these growth parameters represent the remaining fish that were targeted. In this case, the effects of size-selective fishing do not need to be accounted for. However, growth parameters describing the harvested targeted population will change as vulnerability changes (as fishers target smaller fish, for example). If a researcher wishes to estimate the potential growth under no harvesting, then growth parameters representing the entire population are needed.

Recently, Laslett et al. (2002) derived a likelihood function for analysis of growth data from tagging experiments that accounts for individual variation in growth through variation in individual asymptotic lengths L_{∞}, measurement errors, and possible changes in the von Bertalanffy metabolic parameter K with fish age. Unfortunately, they follow previous workers (James 1991; Palmer et al. 1991; Wang et al. 1995) in assuming away one of the most common sources of bias in growth data; they assumed independence of sampled individual L_∞ and age despite much evidence that higher L_∞ (fast-growing) individuals have a higher capture probability in fishing and sampling gear used to collect the length–age samples.

To deal with nonindependence of age and individual L_{∞}, likelihood functions for the analysis of length–age samples should include parameters representing probabilities of capture-at-age and probabilities of capture-at-length (i.e., size selectivity). Where applicable, the cumulative effects of size-selective harvesting on apparent growth should also be considered. In this paper, we derive likelihood functions that include size selectivity, mortality, and growth parameters based on the assumption that size–age data are sampled from a multinomial distribution. We derive four likelihoods: (*i*) a full likelihood and (*ii*) a "reduced" likelihood using the conditional maximum likelihood estimates for numbers-at-age instead of estimating natural mortality M, (*iii*) a full likelihood that includes cumulative effects of fishing mortality F, and (*iv*) a reduced likelihood with cumulative effects of F. We test the performance of these formulations with 100 simulated data sets and show that they are better estimators of growth parameters than previous statistical models for growth data. Finally, we fit all four models to real length-at-age data from the pigmy northern pikeminnow (*Ptychocheilus oregonensis*) of south-central British Columbia.

Methods

Likelihood derivation

We assume that the basic data available for analysis consists of a matrix $n_{l,a}$ with elements of numbers of fish sampled at (discrete) lengths l and ages a and that this is a random sample of the number of vulnerable fish of length l and age a available in the population to be sampled. In this case, the general form of the likelihood for $n_{l,a}$ given the parameter vector Θ is multinomial, and the log likelihood is the product of the data $n_{l,a}$ and the log of the predicted proportions $p_{l,a}$ of length l and age a:

$$(1)\qquad \ln L_1(n|\Theta) = \sum_l \sum_a n_{l,a} \ln(p_{l,a})$$

How the $p_{l,a}$ term of the likelihood is computed depends on which of the four likelihoods presented below is being used.

Model 1: growth with no history of fishing

Where there has been no history of fishing, we calculate the predicted proportions in the matrix $p_{l,a}$ as the proportion of vulnerable numbers of fish at age a and length l (in millimetres fork length) to the sum across all ages and lengths V_T

$$(2)\qquad p_{l,a} = V_{l,a}/V_T,\ \text{where}\ V_T = \sum_l \sum_a V_{l,a}$$

We begin by assuming that $V_{l,a}$ can be expressed as the product of three factors: a size-dependent vulnerability function v_l that depends on size (but not age), relative abundance N_a of age-a fish, and the conditional probability $P(l|a)$ of being in a discreet length interval l given age a:

$$(3)\qquad V_{l,a} = v_l N_a P(l|a)$$

We assume that v_l has a functional form with increasing vulnerability-at-length:

$$(4)\qquad v(l) = \frac{1}{(1 + e^{-\gamma(l-l_h)})}$$

We describe the length at 50% vulnerability (l_h) as a fraction of L_∞ and a shape parameter (γ) that describes the slope of the curve through l_h (Deriso et al. 1985). If we assume a stable recruitment in the population, then relative numbers-at-age (N_a) can be calculated using

$$(5)\qquad N_a = R\,e^{-M(a-1)}$$

Equation 5 computes the exponential decay of older cohorts relative to R individuals. The natural mortality rate M is estimated with the growth and vulnerability parameters using eq. 1. Where the assumption of a stable recruitment fails, it can be relaxed in the reduced likelihoods described below.

If we assume that variation in growth among individuals of age a is caused mainly by variation in individual asymptotic lengths $L_{\infty,i}$, then the von Bertalanffy prediction of size-at-age for any individual i is $L_{\infty,i}f(a)$, where $f(a)$ is shared by all individuals i. Using the von Bertalanffy growth model, this shared effect is described by

$$(6)\qquad f(a) = 1 - e^{(-K(a-t_0))}$$

where K is the metabolic growth parameter and t_0 is the theoretical time of zero length.

Assuming that individuals with $L_{\infty,i}$ present at the start of cohort life are drawn from a normal distribution with mean L_∞ and variance σ^2_∞, a subcohort of a specific $L_{\infty,i}$ will follow a growth trajectory determined by the $L_{\infty,i}$ that they were born with. This implies that the integral defining $P(l|a)$ for each age a should be evaluated with mean $l_a = L_\infty f(a)$ and variance in mean length-at-age σ^2_a:

$$(7)\qquad P(l|a) = \frac{1}{\sigma_a\sqrt{2\pi}}\int_{l-d}^{l+d}\exp\left[\frac{(l_a - l)^2}{2\sigma^2_a}\right]dl$$

where l is the length of the fish, d is half of the length interval width, l_a is the mean length-at-age, and σ^2_a is the variance in length-at-age. In most species of fish, the absolute value of σ^2_a increases with age σ^2_a (gets larger as a increases). To avoid estimating σ^2_a for each age, we assume that standard deviation at age σ_a can be expressed as a simple function of the mean length-at-age l_a. This function can be l_a multiplied by some coefficient of variation (CV), which is assumed to be constant across all ages or more complicated functions can be used. We chose to use eq. 8 derived by Fournier et al. (1991):

$$(8)\qquad \sigma_a = \lambda_1 e^{\lambda_2\left(-1+2\frac{1-\rho^{a-1}}{1-\rho^{A-1}}\right)}$$

In this formulation, ρ is the Brody growth coefficient ($\rho = e^{-K}$), A is the number of ages age a, λ_1 represents the magnitude of the standard deviations at age a, and λ_2 determines the length-dependent trend in the standard deviations (if $\lambda_2 = 0$, the standard deviations are independent of length) (Fournier et al. 1991). This formulation has the disadvantage of adding two parameters over one if σ^2_a is expressed as CV × l_a. The rational for using it, however, is that it makes a less restrictive assumption about how σ_a changes as fish grow older. It also reduces the covariation in the parameters by making that standard deviation of length-at-age depend on one growth parameter ($\rho = e^K$) rather than all three as it would if it were expressed as l_a × CV.

Model 2: reduced likelihood

An option for avoiding the assumption of stable recruitment is to construct the model using the conditional maximum likelihood estimates for numbers-at-age $\hat{N}_a$ and using these in the predicted sample proportions-at-length and -age ($p_{l,a}$) instead of the N_a predicted by eq. 5. This relaxes the assumption of stable age structure, which would be violated with highly variable recruitment. Differentiating the log likelihood with respect to N_a, setting this derivative to zero, and solving for N_a gives the conditional maximum likelihood estimates $\hat{N}_a$:

$$(9)\qquad \hat{N}_a = \left(\frac{n_a}{n_T}\right)\left(\frac{V_T}{\bar{v}_a}\right)$$

where n_T is the total number aged in the sample, n_a is the total number in the sampled aged a years, and V_T the total vulnerable numbers from eq. 2 now computed without the numbers-at-age term (N_a) and $\bar{v}_a$. The value of $\bar{v}_a$ must first be calculated from the product of vulnerability-at-length and the conditional probability of being in length interval l given age a:

$$(10)\qquad \bar{v}_a = \sum_l v_l P(l|a)$$

Note that this is not a true mean vulnerability-at-age. It represents the sum vulnerabilities-at-length in each age-class weighted by $P(l|a)$.

If the prediction of N_a from total mortality rate (eq. 5) is replaced in the likelihood by these conditional maximum likelihood $\hat{N}_a$ estimates (allowing for any possible age structure in the population being sampled), the multiplicative term n_a/n_T, which does not vary with the parameters, can be dropped and $p_{l,a}$ expressed as

$$(11)\qquad p_{l,a} \propto \frac{v_l P(l|a)}{\bar{v}_a}$$

instead of eq. 2, so the reduced likelihood (L_2) to be maximized without M becomes

$$(12)\qquad \ln L_2 \propto \sum_l\sum_a n_{l,a}\ln\left(\frac{v_l P(l|a)}{\bar{v}_a}\right)$$

Model 3: growth under harvesting

To model the effects of growth under harvesting, we again take the approach that $n_{l,a}$ is a random sample from a population where $V_{l,a}$ is the total number of vulnerable fish of length l and age a. The same general form of the likelihood function (eq. 1) applies but a historical fishing rate (F) must now be incorporated into the estimated sample proportions $p_{l,a}$. To do this, we multiply each $V_{l,a}$ element by a length- and age-specific survivorship $\ell_{l,a}$ that accounts for a growth pattern that subjects individual fish to both fishing mortality and natural mortality.

Absent temporal information on past variation in recruitment, natural mortality rate M, and overall (fully vulnerable ages) fishing mortality rate F, we treat these factors as having been stable for enough years to result in a stable age–size distribution. When a population has been subjected to a fishery, the numbers at each age cannot be described by eq. 5 because each individual fish will be subject to a fishing mortality dependent on the growth trajectory of that individual. In this case, the N_a component of $V_{l,a}$ in eq. 3 becomes $N_{l,a}$, which must be computed for each l and a combination. The $N_{l,a}$ represents the survivors of fish that had individual $L_{\infty,i}$ equal to $l/f(a)$, i.e., followed a growth pattern that subjected them to cumulative instantaneous mortality (now including the fishing mortality F) at length and age:

$$(13)\qquad Z_{l,a} = \sum_{a'}[M + v_{l/a'}F]$$

to give

$$(14)\qquad N_{l,a} = Re^{-Z_{l,a}}$$

where the sum (or integral) over a' represents mortality over ages up to age a and where $v_{l(a')}$ represents the time sequence of v_l vulnerabilities seen by fish that followed the growth trajectory $l_{a'} = L_{\infty,l}f(a') = [l/f(a)]f(a')$ over ages a'. The initial recruitment can be set to $R = 1$ for convenience, since only the ratios of the $V_{l,a}$ to V_T appear in the likelihood function (the composition information $n_{l,a}$ carries no direct information on total population size). For typical vulnerability functions $v(l)$ describing v_l, there is no simple analytical solution for the sum or integral of $v_{l(a')}$ values over ages a', but these sums can be easily evaluated numerically given any assumed form for $v(l)$. This integral needs to be evaluated for all l, a combinations, since each such combination is assumed to have had a different $L_{\infty,l}$ and hence a different exploitation history. With a history of exploitation, the vulnerable numbers at age and length $V_{l,a}$ are calculated as

$$(15) \quad V_{la} = v_l R e^{-Z_{l,a}} P(l|a)$$

The $p_{l,a}$ in eq. 1 is now calculated with $V_{l,a}$ calculated using eq. 15:

$$(16) \quad p_{l,a} = \frac{V_{la}}{V_T} = \frac{v_l R e^{-Z_{l,a}} P(l|a)}{V_T}$$

so that the full likelihood (L_3) that includes size-selective fishing mortality is

$$(17) \quad \ln L_3(n|\Phi) = \sum_l \sum_a n_{l,a} \ln\left(\frac{v_l R e^{-Z_{l,a}} P(l|a)}{V_T}\right)$$

The only numerically complex part of this growth under fishing formulation is computing the length–age-specific survivorship ($e^{Z_{l,a}}$) for each l, a element. Consider, for example, the function $v(l)$ in eq. 4. Representing $l(a')$ as $l(a') = L_{\infty,l}f(a')$, this age function can then be substituted for lengths in the vulnerability function for all length intervals l. For example, the substitution for the vulnerability function in eq. 4 is

$$(18) \quad v(a') = \frac{1}{\left(1 + e^{-\gamma(f(a') - l_h/L_{\infty,l})}\right)}$$

which then has to be integrated over a' and then multiplied by F to predict cumulative fishing mortality effects on the "subcohort" of fish that started life with asymptotic size $L_{\infty,l}$. For alternative vulnerability functions that are difficult to integrate (i.e., dome-shaped curves), we recommend simply summing the function over ages a' from 1 to a using a' age steps of 1 year for long-lived fish and shorter steps (e.g., 0.5 year) for fish that live only a few years.

Model 4: reduced likelihood with fishing

To compute the reduced likelihood under fishing for the growth under harvest scenario we need to include the individual the length–age specific survivorship into the calculation of eq. 10 to give:

$$(19) \quad \tilde{V}_a = \sum_l v_l R e^{-Z_{l,a}} P(l|a)$$

Table 1. Parameters used to generate fake data.

Parameter	Value
N_T	1000
$\ln L_\infty$	5
K	0.15
t_0	–0.25
Z	0.2
l_h	0.6
γ	0.1
λ_1	16
λ_2	0.5
CV_R	0.5

Once this is done, $\tilde{V}_a$ gets updated automatically with changes with F. Note that M still appears in eq. 13 but does nothing to change the likelihood, so is not estimated. The $p_{l,a}$ matrix now is computed as

$$(20) \quad p_{l,a} = \frac{v_l R e^{-Z_{l,a}} P(l|a)}{\tilde{V}_a}$$

and the reduced likelihood now including fishing becomes

$$(21) \quad \ln L_4 = \sum_l \sum_a n_{l,a} \ln\left(\frac{v_l R e^{-Z_{l,a}} P(l|a)}{\tilde{V}_a}\right)$$

Simulations

To test the derivations above, we simulated data using known parameters (listed in Table 1). Variation in past recruitment anomalies was included as lognormal process error with a mean of 0 and standard deviation of 1 multiplied by a coefficient of variation, CV_R.

We compared the performance of the standard Fabens (after Fabens (1965)) method that assumes a representative sample of numbers-at-age and minimizes the sum of squares difference between predicted and observed lengths-at-age to the four models that we derive above using simulated data. We generated 100 data sets with a no fishing ($F = 0$), with a sustainable fishery ($F = K$), and an overfished population ($F = 1$). This allows comparison of the estimated parameter values with those used to simulate the data.

Fisheries where the fishing mortality has been stable for a long time are uncommon. Using these same parameters (listed in Table 1) and a fully length–age-structured simulation model, we tested models 3 and 4 with simulated data from stocks where F increased in increments of 0.1/year for 5 years and where F was increased in increments of 0.1/year for 10 years. To do so, new recruits each year were assumed to have a fully representative distribution of individual asymptotic lengths (with mean $L_{\infty,l}$ and variance σ_∞ computed with eq. 8 evaluated at an infinite age). Individuals of specific asymptotic lengths ($L_{\infty,l}$) across all ages were then exposed to fishing mortality every year according to the size (and hence v_l) that they were in that year. As in the simulations with stable fishing mortality, natural mortality is assumed constant and CV_R set to 0.5.

The sensitivities of all of the models to changes in parameters used to simulate data were tested by using extreme values. We conducted simulations with the length at half vulnerability l_h set to 0.05 × L_∞ and 0.9 × L_∞, knife-edged vulnerability (γ = 0.9), and with low variability in length-at-age (λ_1 set to 5). Finally, we tested the models with a short-lived, fast-growing population with K = 0.7 and M = 0.7.

Growth parameter estimation for northern pikeminnow

We collected data for northern pikeminnow from Moose Pasture Lake located on the Bonaparte Plateau approximately 100 km north of Kamloops, British Columbia. We sampled during a depletion experiment in which fish were collected for aging. Fish were captured during six bouts of fishing using four hoop nets. Three of these nets consisted of 6 m × 1 m fiberglass hoops covered in 1-cm mesh with a 20-m center lead and 15-m side leads. The other consisted of 6 m × 0.7 m steel hoops covered in 0.5-cm mesh with a 15-m center lead and 10-m side leads. Lapilli otoliths were taken from each fish and 945 were aged. We cut larger otoliths along the ventral/dorsal axis using an Isomet Bueller slow-speed saw and burned and counted annuli. Otoliths too small to cut were burned and counted directly. For fish older than 5 years, failing to cut otoliths along the long axis resulted in significant underestimates of age.

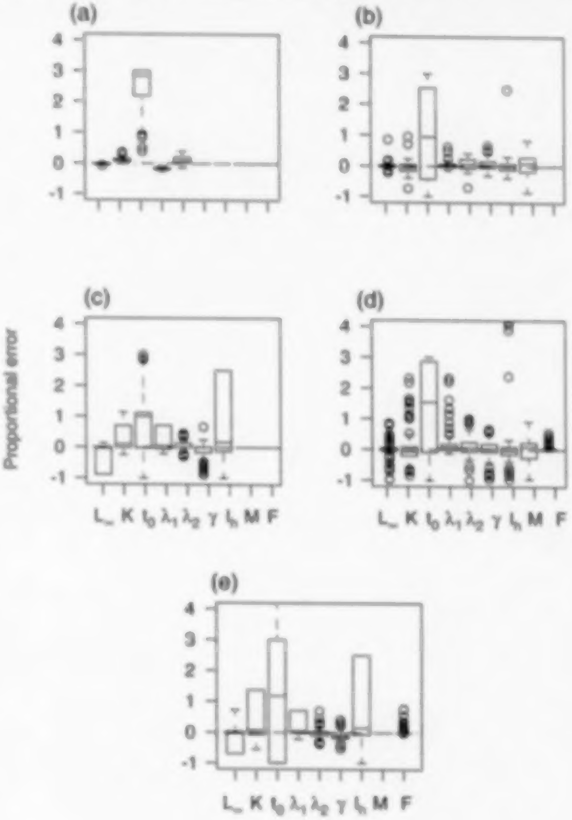
During the 3 months preceding the depletion (9–15 September 2002), 1004 fish were tagged using 5- and 15-mm Floy numbered tags during four bouts of mark–recapture conducted at approximately 2-week intervals. Only those fish that were released in perfect condition are included in this analysis. Because the functional form of the vulnerability-at-length is unknown for fyke nets and northern pikeminnow, we used these tagging data to directly estimate vulnerabilities-at-length. We divided the fish into 5-mm size bins and estimated the gear selectivity for each bin using the likelihood described by Myers and Hoenig (1997). We include this analysis both to ensure that the functional form of the vulnerability-at-length has been specified correctly and to compare how well the vulnerability function estimated using only the length-at-age data and how the multinomial likelihood(s) performs relative to the direct estimates of vulnerability computed from the tagging experiment.

Results

With no fishing mortality F, K was overestimated and L_∞ underestimated using the Fabens method (Fig. 1). These biases were small (0.20 for K and negligible for L_∞). There was still the common bias of a small t_0 parameter, which was underestimated by a factor of 2. With no fishing, models 1, 2, 3, and 4 were unbiased for the growth and vulnerability parameters but slightly overestimated natural mortality M and F.

The bias of Fabens and models 1 and 2 increased with increasing F. With the Fabens method, the bias was strong for t_0, small for K, and negligible for L_∞ (Fig. 1). Models 1 and 2 still performed reasonably well. Model 1 accounted for F by overestimating M (Fig. 2) but could not account for the selective mortality on higher $L_{\infty,i}$ individuals caused by fishing and so underestimated L_∞. Although model 2 suffered from the same bias in growth parameter estimates as model 1 did, models 3 and 4 were unbiased.

Fig. 1. Proportional error in parameter estimates for each likelihood formulation with F = 0. (*a*) Fabens; (*b*) model 1; (*c*) model 2; (*d*) model 3; (*e*) model 4. Circles represent outliers.



Biases in parameter estimates were much worse where F = 1.0 for all but models 3 and 4 (Fig. 3). The bias in K, L_∞, and t_0 was less severe for models 1 and 2 but in the same direction as Fabens. When F was low, the distortion in the sample caused by F was compensated for by high estimates of M (Fig. 2). This was not the case with high F (Fig. 3). The F distorted the sample so much that the few older fish left were the small $L_{\infty,i}$ (slow-growing) individuals.

Models 3 and 4 correctly estimated the von Bertalanffy growth parameters (Fig. 1) at all levels of stable F. Even with moderate recruitment anomalies used to simulate the data (CV_R = 0.5), F was overestimated. This was the case at low F (although it cannot been seen because of the scaling of Figs. 1 and 2) and much worse at higher F (Fig. 3).

The performance of models 3 and 4 suffered with variable fishing. All of the parameters were estimated well with 5 years of increased fishing (Figs. 4*a*, 4*c*, and 4*e*). With 10 years of increased fishing, model 5 (Fig. 4*f*) performed as poorly was Fabens (Fig. 4*b*) and model 4 slightly better (Fig. 4*d*).

Fig. 2. Proportional error in parameter estimates for each likelihood formulation with $F = 0$. (*a*) Fabens; (*b*) model 1; (*c*) model 2; (*d*) model 3; (*e*) model 4. Circles represent outliers.

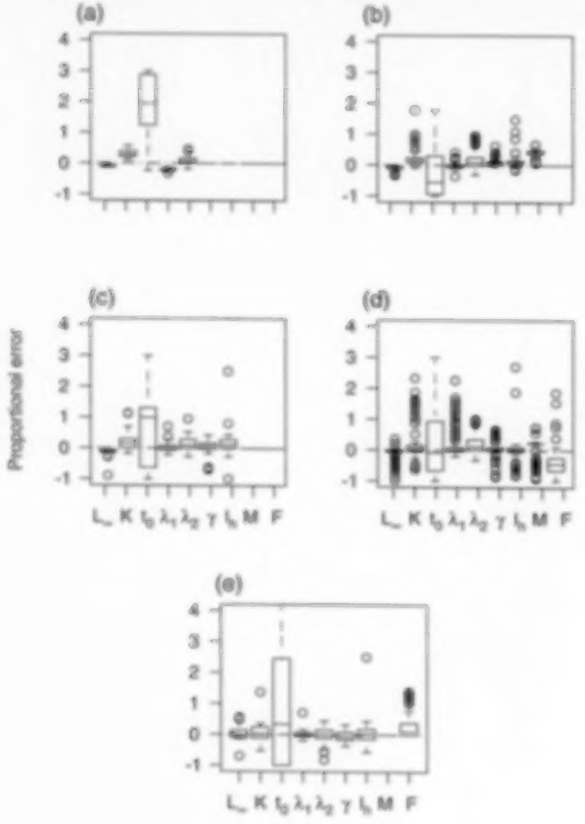
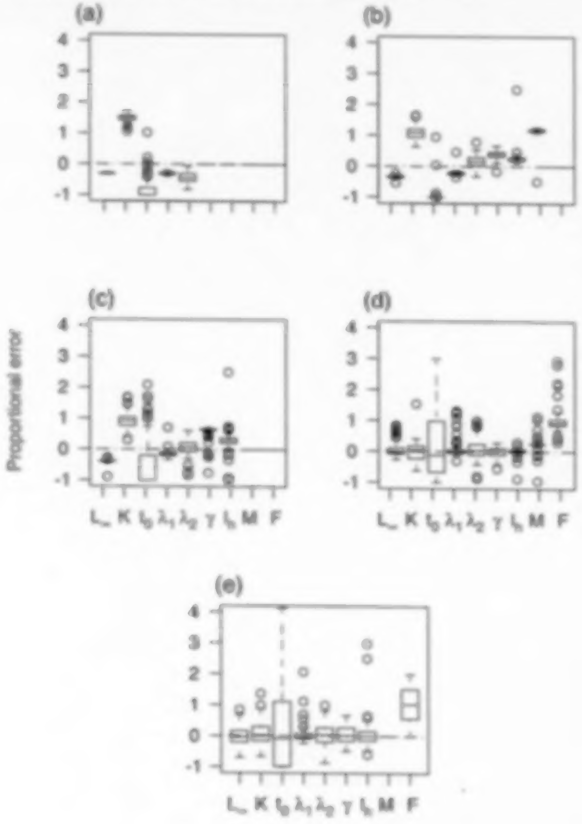


Fig. 3. Proportional error in parameter estimates for each likelihood formulation with $F = 0$. (*a*) Fabens; (*b*) model 1; (*c*) model 2; (*d*) model 3; (*e*) model 4. Circles represent outliers.



At all levels of fishing, models 2 and 4 were less precise than models 1 and 3. Models 2 and 4 do not assume a stable age distribution, and so, more parameter combinations can explain the data. The reduced likelihoods effectively estimated recruitment of each age-*a* cohort $R_t - a$. This means that *a* additional parameters are estimated. While there is loss in precision of the parameter estimates, it is a much more honest acknowledgement of the uncertainty of them in cases where a stable age structure is doubtful or unknown.

All of the models are sensitive to low values of l_h. A very slowly sloping vulnerability ($\gamma = 0.05$) function had the same effect. Distortion in the data of younger age-classes relative to fully represented ones needs to be present in the data to resolve the vulnerability parameters. For example, with no fishing, using the same growth parameters listed in Table 1 but with an l_h equal to $0.05 \times L_\infty$, the data do not contain any information about the vulnerability parameters with mean biases of 3 for l_h and 5.1 for γ. The M is confounded with the vulnerability parameters and is underestimated by 0.28. Fortunately, the growth parameters K, L_∞, and t_0 are well estimated with a mean bias of less than 0.05. As expected, with little size selectivity, the Fabens method actually performs as well as the likelihoods presented here because there is not significant distortion in length–age samples from either fishing or gear selectivity.

With no fishing and parameters again set to those listed in Table 1 but making $l_h = 0.9$, the problem is that there are no fully represented age-classes in the length–age sample. The parameters L_∞, K, λ_1, λ_2, and l_h are all well estimated with biases less than 0.08, but t_0, γ, and M are biased by 0.35, 0.21, and −0.40, respectively. As long as l_h is an intermediate value, then the model is robust to steeply sloping v_l ($\gamma = 0.9$).

The models were robust to data simulated with faster growing (moderately high $K = 0.5$) and faster dying (higher $M = 0.6$) populations, but in populations where K and M are greater than about 0.7, fractional (half or quarter year) ages need to be used or the model performs badly and either underestimates K or fails to converge at all.

These models break down when the shape of the vulnerability curve is not specified correctly, if F is too large, or if M is size or age dependent and with smaller length-at-age samples. If the actual vulnerability function is dome shaped but an asymptotic function is assumed in the analysis, F is confounded with decreasing vulnerability of older/larger fish and becomes biased upward. If F is very high, it is very de-

Fig. 4. Proportional error of (*a* and *b*) Fabens, (*c* and *d*), model 3, and (*e* and *f*) model 4, where F increases in increments of 0.1 to (*a*, *c*, *e*) $F = 0.5$ and (*b*, *d*, *f*) $F = 1.0$; F was estimated but the proportional bias is not included here for clarity. Circles represent outliers.

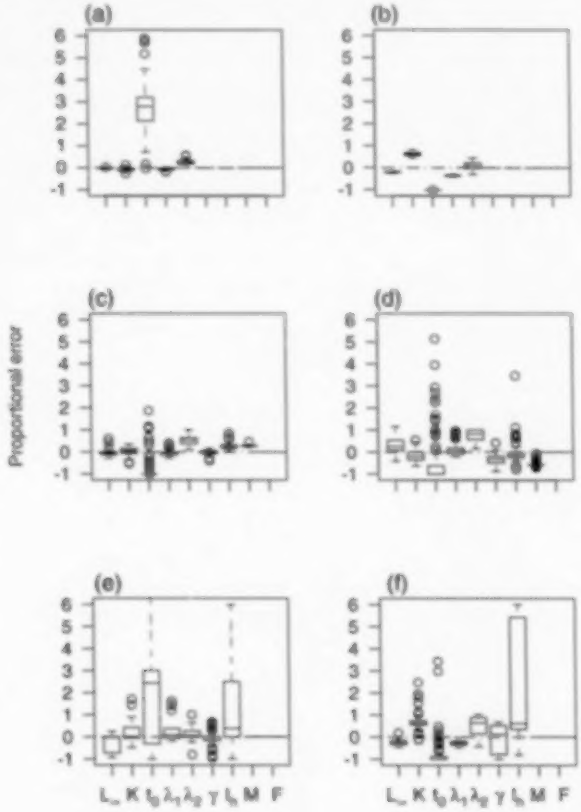


Fig. 5. Growth curves fit to northern pikeminnow data using all five models. Solid, Fabens; short-dashed, full likelihood; dotted, reduced likelihood; dot-dashed, full likelihood with fishing; long-dashed, reduced likelihood with fishing.

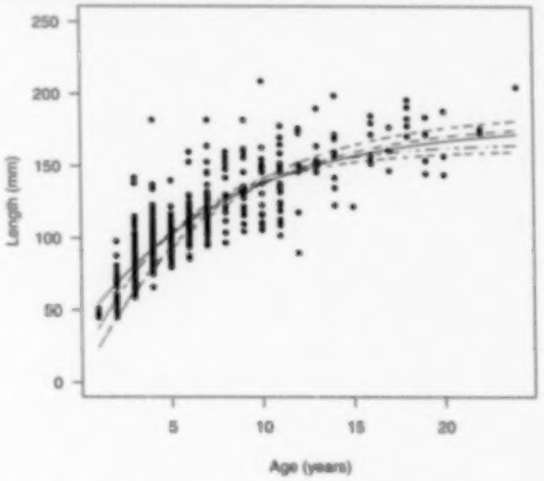


Table 2. Estimated parameter values for each model.

	Model				
Parameter	1	2	3	4	5
L_∞	179.61	162.06	179.77	166.46	188.10
K	0.12	0.19	0.14	0.18	0.14
t_0	−1.95	−0.41	−0.00	−0.38	−0.00
λ_1	13.90	13.18	16.16	13.20	15.96
λ_2	0.69	0.65	0.60	0.61	0.57
l_h	—	0.45	0.49	0.44	0.47
γ	—	0.21	0.20	0.19	0.19
Z	—	0.38	—	0.26	—
F	—	—	—	0.18	0.15

structive to the age structure of the population and no parameters can be estimated well. Where M is size or age dependent (where M decreases over age, for example), there is severe confounding of M and F with the parameters that describe v_j. At low sample sizes, it is possible to have no data by random chance from either the faster growing younger fish (that help resolve v_j) or older fish (that resolve M and F). With 25 age-classes, these models worked very poorly with fewer than approximately 500 samples.

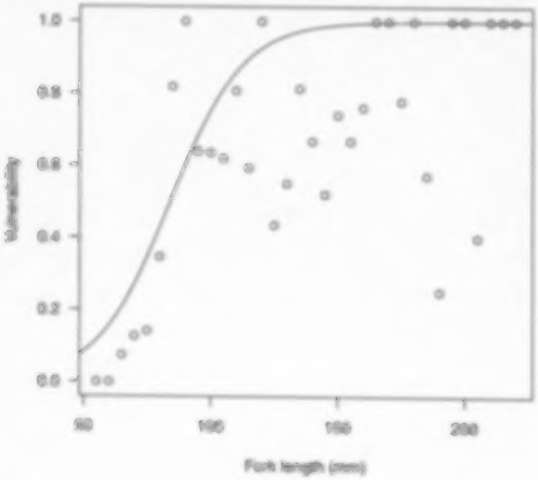
Choosing between reduced or full likelihoods can be done empirically using various information criteria (Bayesian, Akaike, etc.). When the Akaike information criterion was used on simulated data, the reduced likelihood was almost always favored because even with the added number *a* estimated parameters, the value of the objective function at the minimum log likelihood was generally about three orders of magnitude smaller, thus overpowering any penalties for additional parameters. However, when the stock assessment scientist has some prior knowledge of past recruitment anomalies and mortality from the stock assessments, these data could simply be included as terms in the likelihood calculation.

Northern pikeminnow growth

Each of the five model fits to the northern pikeminnow data is plotted in Fig. 5. Parameter estimates are very similar for all of the models used. The likelihood formulations that include fishing (models 3 and 4) actually estimate a small F in this population. Models 4 and 5 have a tendency to overestimate F, but a nonzero value for this parameter is not as unusual as it might seem. The population is exposed to a small-scale fishery from local lodge owners who have in the past conducted annual trapping programs during the spring spawn.

Parameter estimates from all four models are very similar (Table 2.). The most reasonable ones to choose, however, are those fit using model 5. We have no prior knowledge of the M of the population or of the stable age structure in the past.

Fig. 6. Plot of vulnerability curve (line) for northern pikeminnow estimated using model 5 and direct estimates of vulnerability using mark–recapture.



The vulnerability curve estimated by model 5 (Fig. 6) is very similar to that fit using the mark–recapture data. Fitting with model 5 appears to overestimate the vulnerability-at-length for smaller size-classes (Fig. 6) but otherwise seems to be a reasonable approximation. Note that the vulnerability-at-length for the higher size-classes is not well defined because of the small sample sizes of tagged animals in those size bins.

Discussion

The estimated L_∞ of northern pikeminnow is tiny compared with other documented populations. While the von Bertalanffy growth parameter K for this population of northern pikeminnow is similar to those published in the literature, the L_∞ is much smaller. For this reason, we have dubbed this population "the pygmy pikeminnow". Northern pikeminnow have been studied extensively owing to their predation on juvenile salmonids in a variety of systems such as Cultus Lake, British Columbia (Ricker 1941; Steigenberger and Larkin 1974), and they are presently managed in the Columbia basin to reduce their impact on juvenile salmon (Friesen and Ward 1999). Typical L_∞ values for this species are on the order of 400–600 mm total length (Parker et al. 1995) in the Columbia River basin, and in Idaho lakes, the average reported size of a sexually mature female is approximately 440 mm total length (20 in.) for females and 300 mm (15 in.) for males (Jeppson 1959). These values are well above even the maximum sizes that we observe in our study area. The reasons for their small size are presently unknown, but we hypothesize that it is mainly due to the short growing season (3 months or so) that these fish experience.

The simulations shown here are not unusual in showing the biases owing to gear selectivity and size-selective mortality in the estimation of growth parameters. These biases are well known and estimation procedures to deal with them have been developed (Deriso et al. 1985; Parma and Deriso 1990; Sinclair et al. 2002*b*). The methods that we present are original in using simulation studies to show these biases in estimates of the von Bertalanffy parameters and those describing the size selectivity and M and F. Unlike Parma and Deriso (1990) and Sinclair et al. (2002*b*), we make no effort to include environmental effects or, in the case of Sinclair et al. (2002*b*), density-dependent growth on size-at-age. Parma and Deriso (1990) showed that increasing the contribution of environmental factors had little effect on the variance in size-at-age relative to size-selective harvesting and that most fisheries data contain little information about such effects. Sinclair et al. (2002*b*) also determined that by far the largest effect on mean predicted L_∞ was size-selective mortality.

Using our method, we have shown it to be possible under a restricted set of assumptions to estimate the von Bertalanffy growth parameters, M, and F and the vulnerability parameters from only size-at-age data when other data from a fishery are not available. We caution that estimates of the vulnerability and mortality parameters should be considered very uncertain. Unfortunately, the sensitivity of the models to stable F and known vulnerability shape restricts the applicability of these models. Recall that the models were robust to an increasing fishing rate as long as F had not grown too rapidly, while the Fabens method performed almost equally as well in that case but did not tell the researcher anything about M or the vulnerability function v_l. Of particular concern is the breakdown of the models following a very rapid increase in F. Having accurate assessment of the true growth parameters of the stock is particularly needed to assess the rebuilding potential of the stock under rebuilding.

Fortunately, in many cases, the reason for estimating growth parameters in the first place is that it is part of a larger stock assessment where F (and sometimes v_l as in virtual population analysis) is already estimated. This method could be applied in more complex stock assessments where $n_{l,a}$ tables can be provided for a collection of sample years. For each such table, the stock assessment model provides additional information on time-varying recruitments R_{t-a} and F and in some cases (like virtual population analysis) v_l. Assuming that the $n_{l,a}$ data are collected independently each year, the log-likelihood term for each of these tables can simply be added to the overall log likelihood for the assessment model. The accounting (sum or integration) calculations for each $Z_{l,a}$ (cumulative mortality by size and age) would require calculating the survival of those individuals growing along each $L_{\infty,j}$ trajectory but may carry valuable information on changes over time in F. A potentially important advantage of this approach would be to correct the problem that has plagued some past length-based stock assessment methods of ignoring cumulative effects of fishing on length distribution patterns. Mean length and variability of length-at-age are explicitly variable and dependent on F_t in our method owing to variation in $Z_{l,a}$.

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PERSPECTIVE / PERSPECTIVE

Factors for the variability of discards: assumptions and field evidence

Marie-Joëlle Rochet and Verena M. Trenkel

Abstract: Discarding is an issue of increasing concern and there is a growing number of studies aiming at estimating discard amounts and characteristics. However, the sampling design and methods used in these studies generally rely on implicit assumptions. In this perspective, we examine the available evidence in favour of or refuting these assumptions. We find that (*i*) the assumptions most commonly used for estimating discards, namely that discards are proportional to catch or to effort, are generally not supported by the available evidence, (*ii*) both environmental conditions and fishing methods influence the amounts and composition of discards, but because of the huge variability, sampling stratification according to these factors might not result in any improvement of the precision of discard estimates, and (*iii*) many intricate factors can play a role in determining discards in a particular fishery. We conclude that assumptions should be more carefully checked prior to being taken for granted in discard studies and that more studies designed to improve knowledge of the discarding processes are needed.

Résumé : Les rejets font l'objet d'un intérêt grandissant : de plus en plus d'études sont consacrées à l'estimation des quantités rejetées et à leurs caractéristiques. Cependant, les méthodes d'échantillonnage utilizées dans ces études reposent généralement sur des hypothèses implicites. Dans cette perspective, ces hypothèses sont confrontées aux connaissances disponibles et il est établi que (*i*) les hypothèses les plus couramment utilisées pour estimer les rejets, selon lesquelles les rejets sont proportionnels à la capture ou à l'effort, ne sont généralement pas corroborées par les données publiées, (*ii*) les conditions environnementales aussi bien que les méthodes de pêche influencent les quantités rejetées et la composition des rejets, mais une stratification selon ces facteurs n'améliore pas nécessairement la précision des estimations, en raison de la variabilité considérable des rejets et (*iii*) de nombreux facteurs interagissent pour déterminer les rejets dans une pêcherie particulière. En conclusion, le choix des méthodes dans les études consacrées aux rejets devrait être précédé d'une vérification des hypothèses sous-jacentes. Des études des processus conduisant aux rejets sont nécessaires.

Introduction

All animals caught during a fishing operation do not have the same value. Hence, fishers aiming at increasing their revenue will discard the least valuable part of their catch. Fisheries management policies will modify the incentives to discard in various ways (Pascoe 1997). In particular, output controls that limit landings (quotas) or some other characteristics such as minimal landing size will increase the incentives to discard (Pascoe 1997). Therefore, discarding is expected to occur in many fisheries, unless policies specifically aimed at reducing it, such as more selective gears or discard prohibition, are implemented.

Indeed, discarding is an issue of increasing concern, since it was estimated that on average, world fisheries discard almost a third of their total catch (Alverson et al. 1994). The emergence of an ecosystem approach as the new paradigm of fisheries management has encouraged both scientists and managers to give greater consideration to this hidden side of fishing activities (e.g., Kaiser and de Groot 2000; Commission of the European Communities 2002; Pitcher et al. 2002). The number of entries in the international bibliographic database "Aquatic Sciences and Fisheries Abstracts" comprising "discards" or "discarding" in their title has increased from one or two per year in the 1980s to five to 10 in the 1990s and 15 to 19 in 2000–2002. The bulk of these papers are descriptive and primarily aim at estimating the amount or proportion discarded as well as the species and length compositions of discards. The utilization of these estimates is slowly turning from improving the estimates of

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catch and fishing mortality rates in single-stock assessments to assessing the impact of fishing on fish communities. One of the difficulties in estimating discards is their high variability between fisheries, gears, ports, seasons, years, areas, trips, and hauls (see all references below). This variability causes sampling programs to be very expensive, even to achieve a relatively low precision (e.g., Allen et al. 2002; Rochet et al. 2002). A variety of methods have been suggested to limit these costs by making use of additional information, e.g., stratified sampling (Stratoudakis et al. 1999; Tamsett and Janacek 1999), sampling proportional to vessel size (Allen et al. 2001; Cotter et al. 2002), ratio estimators based on landings (Saila 1983), and length structure based (Macer and Brown 1987; Pálsson 2003) or species composition based methods (Medley 2001). Some of these methods are widely used (see below). However, all of these methods rely on implicit assumptions about the factors determining discards variability. This perspective reviews the assumptions concerning the driving factors of discards, examines their theoretical foundations, and compiles evidence supporting or refuting them.

Time has passed since Ray Hilborn deplored the absence of theory, which was depriving the developing discard studies of any direction (Hilborn 1985). During the 1990s, economic models examining the effects of fisheries characteristics or management policies on the incentives to discard have flourished (most of them are reviewed in Pascoe 1997). However, there still is a gap between these theories and tangible knowledge useful for designing field studies. One reason for this gap is that most economic models depict hypothetical fisheries. Models parameterized on the basis of real data to test predictions, like the work by Vestergaard (1996), are rare, and hence, the predictions derived from these economic models are generally not validated. On the other hand, most field studies are essentially descriptive. Few examine the factors influencing discard variability and those that do generally use exploratory correlation approaches; field studies usually do not consider testing even the most general predictions of economic theory (e.g., de Silva and Condrey 1998; Stratoudakis et al. 1998; Kennelly 1999).

This perspective is intended to contribute to filling the gap by bringing together a very simple theory of discarding and the available evidence emerging from the available discard studies. We begin by outlining a set of assumptions. Some of these assumptions can be tested against field data. Some of them are used in discard sampling and estimation methods. For each assumption, we examine the consistency between the available evidence and the use that is made of it, based on a review of published data and on a case study of discards by French trawlers in the Celtic Sea.

Hypotheses on discarding

A resource, i.e., a fish community living in an environment, is exploited by carrying out a fishing operation, i.e., applying a certain amount of effort with a given fishing gear. This results in a catch, which is the material for the sorting process. Sorting is motivated by economic incentives and both technical and legal constraints. The process is highly complex with many variable components interacting in intricate ways. Each of the elements underlined can be considered as a class of factors that influence discard variability (Table 1).

All assumptions in Table 1 appear to be reasonable. However, making use of any of them in discard sampling or estimation procedures amounts to assuming that the corresponding component has enough influence on the discarding process that it will improve the estimation precision. As the process is multivariate and complex, the relative importance of each factor will probably vary among fisheries and analysis scales. This perspective examines the different hypotheses underlying each class of factors.

Glossary

Some expressions are widely used in discard studies but a clear definition is not always available. We use the following definitions. Discarded amounts are weights or numbers of fish discarded. Discard rates are the ratio of discards to total catch in number or weight. Some authors also use this term for the ratio of discards to landings, which is not a rate. Species composition can be either the list of species found in the discards or the proportion of each species in the discards. Length composition or structure is the frequency of each length-class for a given species. The sorting of the catch of a given species is most of time described by L_{50}, the length at which 50% discarding occurs. A fishing métier is the combination of a fishing gear, fishing area, and target species or group of species.

Assumptions related to resource availability

H1. Environmental conditions are key to discards

The impact of environmental conditions on discard quantity and structure is implicitly assumed in many studies that stratify their sampling design according to area, season, or both (see Table 2 for studies). This assumption generally proved to be true when examined. Season influences discarded amounts in various fisheries (e.g., Liggins and Kennelly 1996; Liggins et al. 1996; Machias et al. 2001), discard species composition or diversity (e.g., Trujillo et al. 1997; Castriota et al. 2001; Stobutzki et al. 2001), and length composition (Stratoudakis et al. 2001). However, given the high variability of discards and the limited amount of sampling effort that can be afforded, the differences among seasons might be too low for the stratification to improve the precision of discard estimates (Stratoudakis et al. 1999). Discards also vary among areas (e.g., Murawski 1996; Stobutzki et al. 2001; Bergmann et al. 2002); depth has been examined in several studies and was generally found to have a significant influence on discard amounts (Moranta et al. 2000; Allain et al. 2003; Sánchez et al. 2004), rates (D'Onghia et al. 2001) and species (Blasdale and Newton 1998; Allain et al. 2003; Sánchez et al. 2004) or length composition (Stratoudakis et al. 1998).

The importance of environmental conditions is also a basic assumption underlying studies designed to identify "hot spots", areas and times of high discard rates (Perkins and Edwards 1996). The ultimate aim of such studies may be to close fishing at these hot spots and thus prevent high discarding. However, high variability may prevent hot spots from being found (de

Table 1. Classes of factors that influence discards and the related assumptions examined in this perspective.

Class of factors	Description	Related hypotheses
Assumptions related to resource availability	Species- or length-composition, year-class strength for a given stock, and the environmental drivers of resource dynamics (space and time and physical variables) together will determine what is available for the fishing operation	H1. Environmental conditions are key to discards H2. Species composition of communities or length structure of populations determine what is discarded H3. Year-class strength determines discards
Influence of the fishing operation	Amount of time spent fishing, areas fished, and the gear selectivity and mesh size determine which part of the resource comes onboard	H4. Discards are proportional to fishing time H5. Fishing methods determine what is discarded
Catch and discards	Catch is the result of the first two components and should influence small-scale variability in discards. The high spatial and temporal patchiness of fisheries resources will be reflected in the sorting material, whereas the sorting criteria should vary on different scales	H6. Discards are proportional to catch and hence to landings H7. Length composition of the catch determines discard amounts and size
Market incentives to discarding	Market incentives, i.e., the price differential between parts of the catch, are considered by economists as the major cause of discards	H8. Market prices determine sorting criteria
Technical constraints on landings or sorting	Technical factors, mainly the hold capacity and sorting capacity of the crew in space, time, and manpower, will constrain the sorting criteria	H9. Hold capacity limits the storage of fishing products
Regulations influencing discarding	Landing parts of the catch can be prohibited	H10. Minimum landing size determines the maximum length of discards H11. Total allowable catch and quotas create incentives for discarding

Silva and Condrey 1998) or make the subsequent management measures difficult to implement (Kennelly 1999).

H2. Species composition of communities or length structure of populations determines what is discarded

Medley (2001) developed a method to estimate discards based on the assumption that species composition of the catch should reflect species abundance in the fished community. Although many studies report on the diversity and species composition of discards, we are aware of no study attempting to relate community species composition (e.g., obtained from scientific surveys) to the species discarded. Similarly, the relationship between survey-based length composition of a given species and the discards of this species by commercial vessels has not been examined, although several methods to estimate discards based on research vessel trawl survey catches and selectivity data assume such a relationship (Mayo et al. 1981; Casey 1996).

H3. Year-class strength determines discards

A related assumption is that for commercial species, mainly recruits are discarded; hence, year-class strength should be reflected in the amounts of small fish discarded. Investigating this hypothesis relies on the availability of time series of both discards by species and estimates of recruits, which are obtained from either scientific surveys or stock assessments. These time series are not available for many fisheries. When examined, this was generally found to be true (Reeves 1990; Weber 1995; see below) but not for North Sea cod (*Gadus morhua*) discards by shrimp trawlers (Revill 1997).

Influence of the fishing operation

H4. Discards are proportional to fishing time

A widely used assumption in discard estimation procedures is that discards are proportional to the amount of effort, usually measured in time spent fishing. This assumption is based on the idea that (*i*) catch would be proportional to effort and (*ii*) discards are proportional to catch. The latter assumption will be examined below (H6). That catch is proportional to effort might not always be true, either at the haul level (Gillis 1999; Somerton et al. 2002) or on a larger time or spatial scale, owing to behavioural reactions of both fish and fishers (Hilborn and Walters 1992). Proportionality of discards and fishing effort is assumed by all authors who estimate discards per unit of effort and measure effort in time spent fishing (see Table 2 for studies). We stress that this is different from estimating discards per tow (e.g., Perkins and Edwards 1996; Gray et al. 2001; Erzini et al. 2002), which usually is a sampling unit and hence implies no particular assumption. The wide use of the assumption that discards are proportional to effort is probably due to the fact that the total number of fishing operations of the fleet is generally unknown; in contrast, an estimate of the total fleet time spent fishing is more readily available. However, discarded amounts and rates have been found to vary nonlinearly with tow duration, independent of the gear used

Table 2. Discarding factors: summary of available evidence from 53 studies.

Hypothesis	Use	No. of studies examining the hypothesis	Evidence	References using the hypothesis
H1. Environmental conditions are key to discards	Widely used for sampling stratification and identifying hot spots	13	Influence generally significant but stratification still inefficient owing to high residual variability	Charuau 1985; Cramer et al. 1995; Stratoudakis et al. 1999, 2001; Ye et al. 2000
H2. Species composition of communities or length structure of populations determines what is discarded	Estimating discards from survey catches	None	—	Mayo et al. 1981; Casey 1996; Medley 2001
H3. Year-class strength determines discards	Predicting discards from recruit indices	4	Significant in 3 studies (groundfish bottom trawl)	None
H4. Discards are proportional to fishing time	Widely used for raising discard estimates	5	Discards depend nonlinearly on fishing time (any gear)	Balguerías Guerra et al. 1993; Stewart and Newton 1993; Cramer et al. 1995; Perez et al. 1995, 1996; Weber 1995; Liggins and Kennelly 1996; Liggins et al. 1996; Trujillo et al. 1997; Dupouy et al. 1998; van Beek 1998; Kennelly 1999; Sartor et al. 2001; Gray 2002; Pierce et al. 2002
H5. Fishing methods determine what is discarded	Sampling stratification and technical regulations	16	Métier, gear, and mesh size significantly influential	Perez et al. 1995, 1996; Trujillo et al. 1997; Stratoudakis et al. 1999; Rochet et al. 2002
H6. Discards are proportional to catch and hence to landings	Most widely used assumption for raising discard estimates	12 (mainly bottom trawl)	Discards: independent of catch (9 studies) and increase nonlinearly with catch (3 studies)	Jermyn and Robb 1981; Stevenson 1983; Briggs 1985; Stewart and Newton 1993; Redant and Polet 1994; Morizur et al. 1996*a*, 1996*b*; Murawski 1996; Kulka 1997, 1998; Walter 1997; de Silva and Condrey 1998; van Beek 1998; Stratoudakis et al. 1999, 2001; Tamsett and Janacek 1999; Ye et al. 2000; Borges et al. 2001; Costa et al. 2001; D'Onghia et al. 2001; Gray et al. 2001; Machias et al. 2001; Monteiro et al. 2001; Pranovi et al. 2001; Erzini et al. 2002
H7. Length composition of the catch determines discard amounts and size	Predicting discards from survey catches plus gear selectivity	5 (bottom trawl)	Catch of smaller fish results in higher discards (2 studies) and smaller sorting size (2 studies)	Powles 1969; Mayo et al. 1981; Casey 1996
H8. Market prices determine sorting criteria	Predicting discards from market prices	7	Low prices result in higher L_{50} in 6 *Nephrops* studies	None
H9. Hold capacity limits the storage of fishing products	Predicting discards from trip duration	4 (bottom trawl)	Proportion discarded increases with trip duration	None
H10. Minimum landing size determines the maximum length of discards	Predicting discards from total catch length distribution and minimum landing size	9	Minimum landing size influential in 6 studies, independent of the type of fishery	Hillis 1981*a*, 1981*b*; Macer and Brown 1987; Pálsson 2003
H11. Total allowable catch and quotas create incentives for discarding	Predicting discards from quota fulfilment	1	Trip quotas change discarding behaviour	None

(e.g., Tamsett et al. 1999; Ortiz et al. 2000; this study). In most cases, discarding increases nonlinearly owing to fish being damaged by long hauls or clogged nets preventing escapement. This supports the alternative hypothesis that discarded biomass is a nonlinear function of haul duration. It might be expected to be true for fragile species such as whiting, which could get squashed by the accumulating biomass in long hauls and therefore be discarded. Hauls with large biomasses could have the same effect. Similarly, overall trip length could influence discarding. Under this hypothesis, discards would be related to trip duration. Several circumstances could lead to this scenario: conservation problems on vessels without freezing facilities or fish species with mainly a market for fresh fish. For example, hake and rays might be concerned by this problem, as they are more perishable than other species.

H5. Fishing methods determine what is discarded

Regulations of gear design and mesh size may be powerful tools for limiting discards (Kulka 1998). Consequently, several studies have examined the hypothesis that the fishing gear, especially its mesh size, the métier, or the characteristics of fishing vessels strongly influence discards. This assumption is also implicit when stratifying sampling design by gear or by métier (see studies in Table 2). Discard rates or amounts and species composition were generally found to vary among gears (Howell and Langan 1987) or métiers (e.g., Perez et al. 1996; Pranovi et al. 2001; Bergmann et al. 2002) or ports (Liggins and Kennelly 1996; Liggins et al. 1996), whereas the vessel characteristics do not seem to be influential (Borges et al. 2001). Mesh size was found to influence the amount (Howell and Langan 1987; Murawski 1996; Gray 2002), diversity (Murawski 1996), and length composition (e.g., Martin and Jean 1958; Stratoudakis et al. 2001) of discards in retrospective correlation-based studies. However, in a study designed for estimating the effect of mesh size on discard rates in a shrimp fishery, discard amounts and proportion were not found to differ among 40-, 50-, and 60-mm stretched mesh sizes (D'Onghia et al. 2001). A study examining the effect of introducing a mandatory grid in the trawls of a shrimp fishery proved that this measure was very efficient in reducing fish discard rates (Kulka 1998). The way the trawl sweeps, interacts with the substrate, and collects debris that obstructs the mesh opening and prevents escapement has also been found to influence discard rates (Howell and Langan 1987).

Catch and discards

H6. Discards are proportional to catch and hence to landings

Discards being proportional to catch is the most widely used assumption for estimating and raising discards: many studies estimate "discard rates", the ratios of discards to landings or to total catch (see Table 2). Some of them use a ratio estimator (Stratoudakis et al. 1999; Ye et al. 2000; Stratoudakis et al. 2001). As in studies estimating discards per unit of effort, the reason for using this method is that the data available to raise the discard estimates to the fleet level are commonly landings. However, every time this assumption has been examined, it has been found that discards were not proportional to the catch or landings of a given species or to total catch. In some instances, discard rates were found to be unrelated to the amount caught (e.g., Reeves 1990; Trujillo et al. 1997; Tamsett et al. 1999); their huge variability at all levels (e.g., Pope et al. 1991; Walter 1997; Allen et al. 2001) makes any attempt to estimate average rates futile. In other cases, the discarded fraction was shown to increase with catch at the haul and trip levels (Evans et al. 1994; Machias et al. 2001; this study). This is probably related to technical constraints (see H9 below).

H7. Length composition of the catch determines discard amounts and size

If length composition of the catch is determining, then more individuals should be discarded when the catch contains more small individuals. Under this assumption, discards could be predicted by deducing length composition of the catch from the length structure in the population and gear selectivity (such as Powles 1969; Mayo et al. 1981; Casey 1996). Amounts discarded were found to be influenced by the amount of small fish in the catch (Jean 1963), average length in the catch at the year level (Reeves 1990), or the size of the smallest fish in the catch at the trip level (Rochet et al. 2002). More surprisingly, L_{50} is larger when larger fish are caught (Stratoudakis et al. 1998; this study), suggesting that sorting criteria depend on the composition of the catch and hence that there exists an expected limited amount to be retained.

Market incentives to discarding

Although often considered as the major factor for discarding, market incentives are seldom considered in discard sampling studies. We did not find any study using economic variables as strata or auxiliary information for estimating discards. Usually the total catch of nonmarketable species is discarded and the whole catch of high-value species is retained, whereas low-value species are partially discarded (Perez et al. 1995; Rochet et al. 2002). However, highly valuable species like maatje herring (prespawning Atlantic herring (*Clupea harengus*)) have not necessarily the lowest discard rates, as for these species, buyers might demand high quality (Pierce et al. 2002). Quality requirements are a cause for discards (Bagge 1981; Perez et al. 1995; Pranovi et al. 2001), but this has seldom been examined quantitatively.

H8. Market prices determine sorting criteria

A few studies report on economic influences on sorting. Market prices were said by fishers to have a stronger influence than minimum landing size on hake and megrim sorting according to a questionnaire survey (Lart 2002). It has been found that market prices influence L_{50} of *Nephrops norvegicus*, with low prices resulting in higher L_{50}, both annually and seasonally (e.g., Hillis 1981*a*; Jermyn and Robb 1981; Macer and Brown 1987). Similar evidence was found for Atlantic cod in northern New Brunswick (Jean 1963). We did not find any evidence for this (see below), maybe owing to examining the question on too short a time scale.

Technical constraints on landings or sorting

H9. Hold capacity limits the storage of fishing products

The corresponding assumption is that the proportion of animals discarded increases with trip duration as storage capacity becomes limiting and is an alternative to assumption H6 above. This has been found to occur in several instances (e.g., Evans et al. 1994; Machias et al. 2001; this study). Another possibility is that when the catch exceeds the sorting capacity of the crew, it is totally discarded. This happens in Mediterranean fisheries for small pelagics (Anonymous 2001). To investigate the hold saturation hypothesis, the ideal sampling scheme includes haul-by-haul sampling of discards and landings. If landings by haul are not available, some insight can be gained by regressing trip discards against trip catch or haul number. However, interhaul catch variability might prevent clear conclusions. Looking at total landings as a function of hold capacity can also provide evidence in support of the hypothesis.

Regulations influence discarding

H10. Minimum landing size determines the maximum length of discards

Minimum landing size is generally thought to be the key to the sorting process: fish smaller than the minimum landing size should be discarded, whereas those larger than the minimum landing size can be retained. As a consequence, the length composition of discards should be derivable from the length compositions of the landings and of a sample from the total catch, assuming that above a certain size (e.g., minimum landing size), discards are nil. This is the basis for a variety of methods to estimate discards by length-class of fish or *N. norvegicus* (see list in Table 2), including nonparametric approaches (Allard and Chouinard 1997). The assumption of minimum landing size being determining has proven to be true in some snapshot studies (Perez et al. 1995; Gray et al. 2001; Gray 2002). In line with this, in medium-term studies, changes in minimum landing size were found to result in changes in L_{50} (Jermyn and Robb 1981; Stratoudakis et al. 1998). If legal mesh sizes are not adjusted to minimum landing sizes, or if mesh size regulations are not complied with, this can result in an increase in the amounts discarded (Charuau 1985). However, in some cases, animals much larger than the minimum landing size have been found to be discarded (Evans et al. 1994; Rochet et al. 2002; see below), suggesting that in these fisheries, the minimum landing size regulation is not effective and that other mechanisms (e.g., market incentives) determine sorting behaviours. In other fisheries, minimum landing sizes are not complied with and animals much smaller than the minimum landing size are retained (Machias et al. 2004).

H11. Total allowable catch and quotas create incentives for discarding

Landings saturation might arise on the fleet level when the total allowable catch is approached. In multispecies fisheries, this will result in all further catch of the concerned species being discarded. This hypothesis has been put forward by Hilborn and Walters (1992) and is thought to be a major flaw of output controls of fisheries (Pascoe 1997). However, it has seldom been examined and we found a single qualitative study supporting it (Perez et al. 1995). Trip quotas were demonstrated to increase incentives to discarding conditional on optimal economic behaviour (Gillis et al. 1995); the authors found some evidence to support their prediction but had few appropriate data. On the other hand, in the shrimp fishery off Newfoundland, a regulation requiring that discarded fish be deducted from the quota was efficient at reducing cod discards (Kulka 1997). The implementation of such a scheme, however, requires a high compliance of fishers or onboard observers as in Canada.

Case study

Materials and methods

We recall and refine the study by Rochet et al. (2002) of the French trawler fleet operating in the Celtic Sea. As a whole, this fleet discarded an estimated 30 000 t in 1997 while landing around 63 000 t. The fleet consists of three métiers, which discarded from around 25% for benthic and gadoid trawlers to 55% for *N. norvegicus* trawlers. These estimates are based on a sample of 462 hauls from 26 trips (see details on sampling design in Rochet et al. 2002). For the present study, data from the 1997 sampling exercise as well as some data for 1984 (Charuau 1985; Biseau and Charuau 1989) and 1991 (Péronnet 1991) were used. We tested all hypotheses for which the appropriate data were available, by métier when possible. Some hypotheses could not be tested, as only discards where sampled by haul, landings being available only by trip. Spearman's rank correlation coefficient ρ was used for testing hypotheses based on positive relationships (Conover 1971). For an easier presentation, we group the results according to what the factors examined are expected to influence: the discard amounts or the sorting criteria (L_{50}).

Factors determining the amounts discarded

H3 (year-class strength determines discards)

For testing the assumption that year-class strength determines discards, we used discard estimates and estimated numbers of recruits from International Council for the Exploration of the Sea working group reports for 3 years for each of 10 species (Rochet et al. 2002). Owing to the shortness of the time series, we pooled across species and standardized both discards and recruits by dividing by the respective mean values per species. A significantly positive relationship was found between standardized discards and standardized recruits (Table 3). This provides evidence in support of the hypothesis of year-class strength influencing discard amounts.

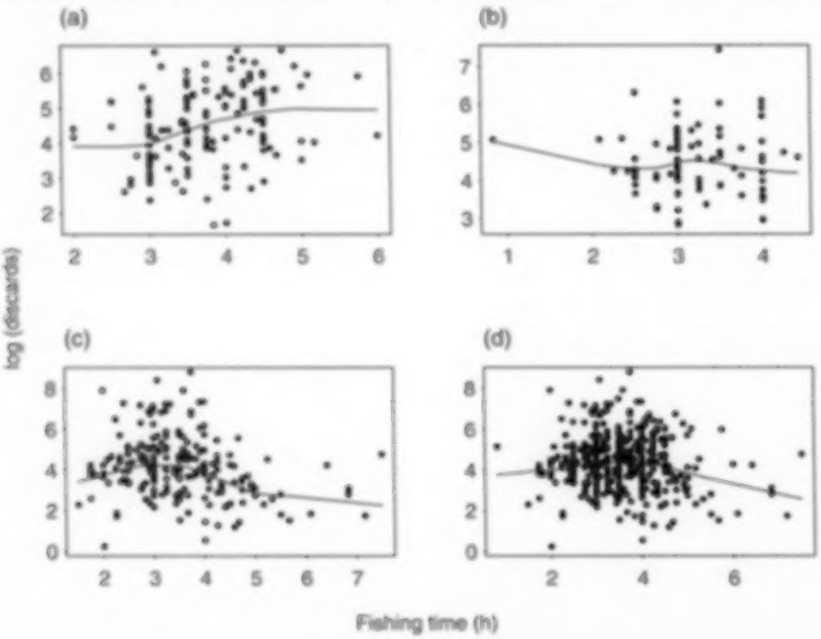
H4 (discards are proportional to fishing time)

Trawl fishing effort at the haul level is measured by the haul duration in hours, while effort at the trip level is expressed as the total trip duration, which includes travelling time. For the Celtic Sea, most sampled fishing trips were of similar length (14 days), thus not providing enough contrast to investigate the impact of trip duration on discarding. In contrast, discards as a function of haul duration were looked at by métier (Fig. 1). Discards increased with fishing time for benthic trawlers but not linearly (hence not proportion-

Table 3. Results of hypothesis tests for the discarding process of French Celtic Sea fleet targeting *N. norvegicus*, gadoids, and benthic species in 1997.

Hypothesis	Test results	Conclusion
Assumptions related to amounts discarded		
H3. Year-class strength determines discards	Slope = 0.3, p = 0.04 (3 years for 10 species)	Evidence
H4. Discards are proportional to fishing time	Benthic: ρ = 0.32, p = 0.0001; gadoid: ρ = –0.03, p = 0.78; *Nephrops*: ρ = –0.21, p = 0.002	Rejected. Benthic: positive relationship (no proportionality); gadoid: none; *Nephrops*: negative relationship (no proportionality)
H6. Discards are proportional to catch and hence to landings; H9. Hold capacity limits the storage of fishing products	$F_{[20,23]}$ = 8.82, p = 0.0006	Proportionality rejected; saturation more probable
H7. Length composition of the catch determines discard amounts and size	$p < 0.05$ for 18 species	Evidence; depends on métier and species
Assumptions related to sorting criteria		
H7. Length composition of the catch determines discard amounts and size	See Rochet et al. 2002	Evidence; differences between métiers and species
H8. Market prices determine sorting criteria	See Rochet et al. 2002	Rejected
H10. Minimum landing size determines the maximum length of discards	See Table 4	Rejected; differences between métiers and species

Fig. 1. Discarded biomass (log (discards (t)) of all species versus haul duration by métier for the French fleet operating in the Celtic Sea during 1997: (*a*) Benthic; (*b*) gadoid; (*c*) *Nephrops* trawlers; (*d*) whole fleet. The lines show a robust local smoother.

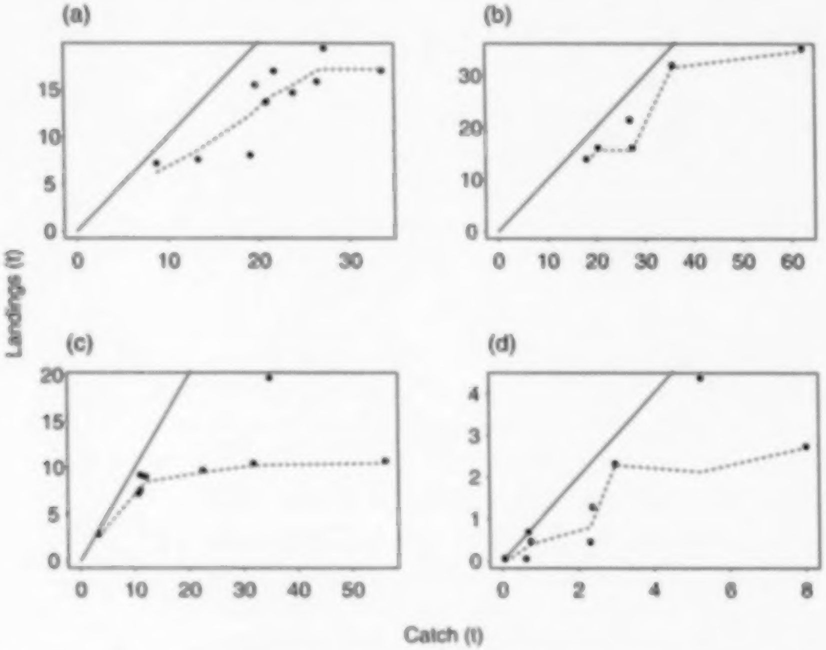


ally). Discards decreased with fishing time for *N. norvegicus* trawlers; there was no clear relationship between discards and effort for gadoid trawlers (Table 3).

H6 (discards are proportional to catch and hence to landings) versus H9 (hold capacity limits the storage of fishing products)

The assumption that trip discards are proportional to catch was evaluated by regressing the discarded amount against total catch. A linear regression passing through the origin (proportionality) was compared with a polynomial regression also passing through the origin (nonlinearity), with separate slopes and curvatures per métier. The assumption of discards being proportional to catch is rejected in favour of a nonlinear relationship, with negative curvatures supporting the idea of a saturation process (Fig. 2; Table 3). Owing to the lack of landings data by haul, this alternative saturation hypothesis could not be formally tested. Instead, we looked

Fig. 2. Total biomass caught and landings per trip (t, tonne) by métier for the French fleet operating in the Celtic Sea during 1997. Total biomass caught and landed by (*a*) benthic, (*b*) gadoid, and (*c*) *Nephrops* trawlers; (*d*) *Nephrops* biomass caught and landed by *Nephrops* trawlers. Points would lie along the continuous line if the whole catch were landed. The broken lines show a robust local smoother.

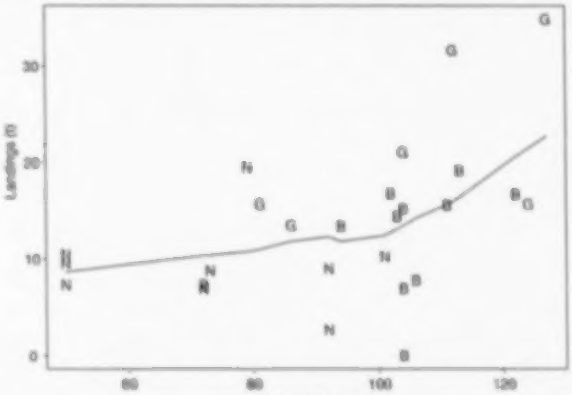


at the relationship between hold capacity and total landings per trip as an indication of saturation (Fig. 3). A positive relationship between the two quantities appeared, providing evidence that hold capacity might play a role in determining total landings and thus discards (Table 3).

H7 (length composition of the catch determines discard amounts and size)

The length of the smallest animal of a given species in the catch was a significant predictor for the amount discarded for all tested species except *N. norvegicus* (Table 3). Full results are reported in (Rochet et al. 2002, their table 9). All species had a negative coefficient, showing that the smaller the animals caught, the more were discarded.

Fig. 3. Trip landings (t, tonne) versus vessel hold capacity (tons; 1 ton = 2.83 m^3) for the French fleet operating in the Celtic Sea during 1997: B, benthic trawlers; G, gadoid trawlers; N, *Nephrops* trawlers. The line shows a robust local smoother.



Factors determining the sorting criteria

H7 (length composition of the catch determines discard amounts and size), H8 (market prices determine sorting criteria), and H10 (minimum landing size determines the maximum length of discards)

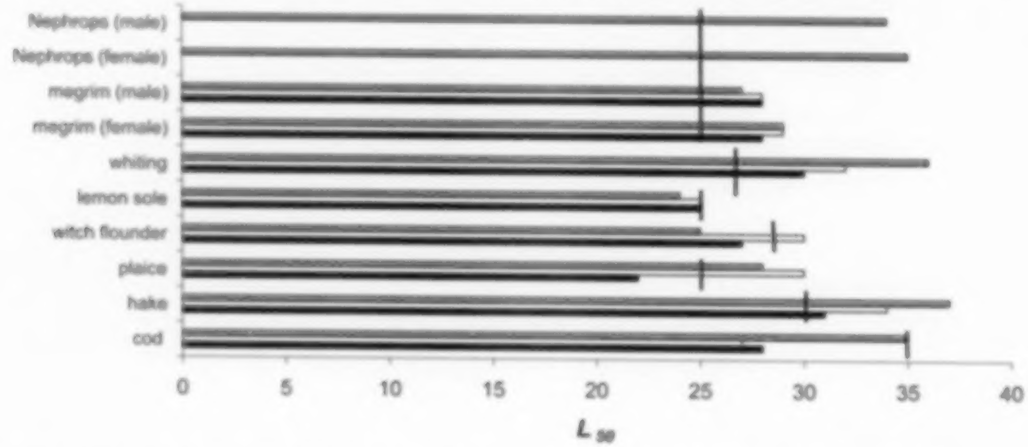
The L_{50} was estimated by fitting separate logistic curves for each of 19 species. Linear models for explaining the estimated L_{50} values per fishing trip for these species were selected by stepwise addition of variables chosen among quartiles of the length distribution of catch by trip, total catch weight of the species, prices of the smallest and largest size categories of the species, trip duration, métier, gear, season, and fishing area. Selected models differed among species, but either minimum or first quartile of the length distribution was the most important explanatory variable, showing evidence in favour of H7. In contrast, prices were never selected, suggesting that H8 should be rejected for this fishery. Median length of discards and estimated L_{50} of nine commercial species (by sex for megrim and *N. norvegicus*), per trip and overall, were compared with the minimum landing size in vigour at the time of the study. It was found that (*i*) for four species, in most trips, most discards were larger than the minimum landing size (Table 4) and (*ii*) overall,

Table 4. Proportion of trips with median length of discards larger than the minimum landing size.

Species	Benthic trawlers	Gadoid trawlers	*Nephrops* trawlers	Whole fleet
Atlantic cod (*Gadus morhua*)	8/9	6/6	4/9	18/24
Female megrim (*Lepidorhombus wiffiagonis*)	5/11	4/6	1/6	10/23
Male megrim	0/9	1/5	1/6	2/20
Hake (*Merluccius merluccius*)	6/10	1/5	0/8	7/23
Whiting (*Merlangius merlangus*)	8/8	3/3	2/5	13/16
Lemon sole (*Microstomus kitt*)	5/9	0/5	0/8	5/22
Female *Nephrops norvegicus*	—	0/1	2/6	2/7
Male *Nephrops norvegicus*	1/1	1/1	4/4	6/6
Plaice (*Pleuronectes platessa*)	5/5	3/6	0/2	8/13

Note: N_1/N_2: N_1 is the number of trips with median length of discards larger than the minimum landing size and N_2 is the number of trips by this fleet with discards of the species.

Fig. 4. Mean length (cm for fish, mm for Nephrops) at which 50% of the fish were discarded by métier: *Nephrops* trawlers (shaded bars), gadoid trawlers (open bars), and benthic trawlers (solid bars). The vertical lines indicate the minimum landing size. Species: megrim (*Lepidorhombus wiffiagonis*), whiting (*Merlangius merlangus*), lemon sole (*Microstomus kitt*), witch flounder (*Glyptocephalus cynoglossus*), plaice (*Pleuronectes platessa*), hake (*Merluccius merluccius*), and Atlantic cod (*Gadus morhua*).



most L_{50} were larger than the minimum landing size (Fig. 4), providing evidence against H10.

Discussion

The assumptions most commonly used for estimating discards, namely that discards are proportional to catch or to effort, are exactly those for which no evidence has been found. Admittedly, this conclusion might be regarded as preliminary, as the number of studies devoted to these questions is modest (17 papers in this nonexhaustive review; see Table 2). These assumptions are convenient in most cases because they provide easy means for raising discard estimates from sampled trips to entire fleets. However, given the evidence against these hypotheses, the resulting estimates are likely to be biased in many cases. We suggest that the alternative assumptions (e.g., nonlinear relationships) be quantitatively examined at all possible scales. Until more conclusive knowledge has been acquired, great care should be taken when interpreting discard estimates obtained using assumptions of proportionality to catch or fishing time. Measuring fishing effort in number of tows rather than time spent fishing and using simple sampling theory, i.e., raising by sampling units rather than using any auxiliary variable, are the recommended methods in the present state of knowledge.

The second group of assumptions that are widely and implicitly used for estimating discards are those underlying the definition of sampling strata. From this review, it is clear that the corresponding assumptions (H1 and H5) are valid and that both environmental conditions and fishing methods influence the amounts and composition of discards. However, this does not automatically mean that stratified sampling is beneficial in all cases. In most instances, the variance part explained by these factors is small, and hence, the remaining variability is still huge. For this reason, stratified sampling might not result in improved precision of discard estimates. Stratification is only useful if it significantly reduces the estimation variance.

All factors identified in this perspective can play a role in determining discards in a particular fishery. In point of fact, the determining factors are expected to vary between fisheries owing to environmental, economic, and social characteristics that will play a major role in the availability of resources and will structure the fishing activity. Therefore, we suggest that pilot studies such as the one by Stobutzki et al. (2001) should be conducted for each fishery to determine the most appropriate raising factors, auxiliary variables, and stratification scheme. For progressing towards a theory of discarding, it would be particularly useful that all discards studies report details about all potentially relevant factors. Metaanalyses of these studies would then be possible. The factors of interest include (*i*) space and time and environmental variables if suspected to strongly influence resource availability, (*ii*) fishing effort: amount and spatiotemporal distribution and gear characteristics, (*iii*) nonaggregated catch statistics to appraise their variability, (*iv*) relative sales value of the catch components, (*v*) hold capacity, trip duration, crew composition, and any constraints on sorting, and (*vi*) current regulations of the fishery: total allowable catches and quotas and legal landing sizes.

Obviously, discarding cannot be considered in isolation from the remainder of the fishing operation. Discarding and the selecting of fishing zones at a given season, also referred to as fishing strategy, are probably closely linked. Babcock and Pikitch (2000) simulated the benefits of different fishing strategies for a US west coast groundfish fishery when total trip landings were limited to actual observations. They concluded that fishers indeed had selected a strategy that guaranteed higher income and incidentally avoided large discards of species for which the trip quota was reached. Not much theoretical or empirical evidence exists to corroborate that the choice of fishing strategies or the related métiers is directly determined by the attempt to reduce or avoid discards of undersized fish, although this can be suspected to be an important factor.

There is a lack of quantitative studies for most of the assumptions regarding discarding behaviours. However, the gap is particularly obvious in the field of market incentives, which are considered by the available theories as the most determining factors for discarding practices. Similarly, the effect of regulations, especially of output controls like quotas, has seldom been examined. These gaps might be due to the difficulty in gathering sufficient data on appropriate time scales. We suggest that studies should be undertaken to further test the assumptions assembled in this perspective to refine a general theory of discarding by identifying the driving factors of discards in relation to the fisheries' characteristics. This task should be easier now that an increasing number of sampling programs are taking place. It is essential that these discard studies be complemented with questionnaire surveys or interviews of fishers regarding their discarding criteria. Interviews are key to ranking factors by influence (Lart 2002) and to understanding discarding behaviours.

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PERSPECTIVE / PERSPECTIVE

Biogeochemistry of persistent bioaccumulative toxicants: processes affecting the transport of contaminants to remote areas[1]

J.M. Blais

Abstract: We release a very wide variety of synthetic chemicals to the environment. The persistence, environmental behavior, and toxicity of these chemicals will determine the severity of their impacts on our ecosystems. In some cases, contaminated areas are far from the source of pollution. The study of contaminants in remote environments, however, provides important information about a chemical's environmental behavior. For example, when anthropogenic chemicals are recorded in very remote arctic environments, we know that these chemicals arrived by long-range transport. When we examine contaminants on a global scale, we observe a global distribution that is not random, but rather represents a systematic geochemical behavior that is affected by atmospheric and ocean transport, deposition from the atmosphere, and concentration in food chains. This paper reviews the nature and processes involved in long-range transport of chemicals to remote environments and explores some of the pathways taken by persistent bioaccumulative toxicants following their release.

Résumé : Les humains relâchent une grande variété de composés chimiques synthétiques dans l'environnement. La persistance, le comportement dans le milieu et la toxicité de ces produits déterminent la sévérité de leur impact sur les écosystèmes. Dans quelques cas, les zones contaminées se situent loin des sources de pollution. L'étude des contaminants dans les milieux éloignés fournit cependant des renseignements importants sur le comportement des contaminants dans l'environnement. Ainsi, lorsque des contaminants d'origine humaine se retrouvent dans des milieux arctiques très retirés, on sait que ces produits chimiques y ont été apportés par un transport sur de grandes distances. L'examen des contaminants à l'échelle globale révèle non pas une répartition aléatoire sur le globe, mais plutôt un comportement géochimique systématique qui est affecté par le transport atmosphérique et océanique, par la déposition atmosphérique et par la concentration dans les chaînes alimentaires. On trouvera ici un examen rétrospectif de la nature du transport de longue distance des produits chimiques vers les milieux éloignés et des processus qui sont impliqués; il y a aussi une exploration de quelques-unes des voies suivies par les produits toxiques bioaccumulables persistants après leur introduction dans l'environnement.

[Traduit par la Rédaction]

Introduction

Persistent bioaccumulative toxicants (PBTs) are a group of chemicals characterized by a high degree of persistence, high toxicity, relative insolubility in water, and a strong tendency to biomagnify in food chains (http://www.pops.int/). The most famous of this group are the organochlorine compounds such as the polychlorinated biphenyls (PCBs), dichlorodiphenyl trichloroethane (DDT), toxaphene, chlordane, lindane, endosulfan, mirex, and the dioxins and furans. Many of these chemicals are now banned or restricted in industrialized countries and are being further restricted in developing countries under the Stockholm Convention (http://www.pops.int/). Nevertheless, their continued presence in the environment still has dramatic health and economic impacts on our society. In 2002, 93% of all >4000 consumption advisories in North America were caused by at least one of the four following PBTs: mercury, dioxins, PCBs, and chlordane (International Joint Commission 2003). All five of the Laurentian Great Lakes currently have fish-consumption advisories for PCBs and dioxins, while four of the five lakes have advisories for mercury and three have advisories for chlordane (International Joint Commission 2003). These advisories are implemented when fish or meat products contain a toxic chemical dose that makes them unsuitable for human consumption. Moreover, sites that were contaminated with

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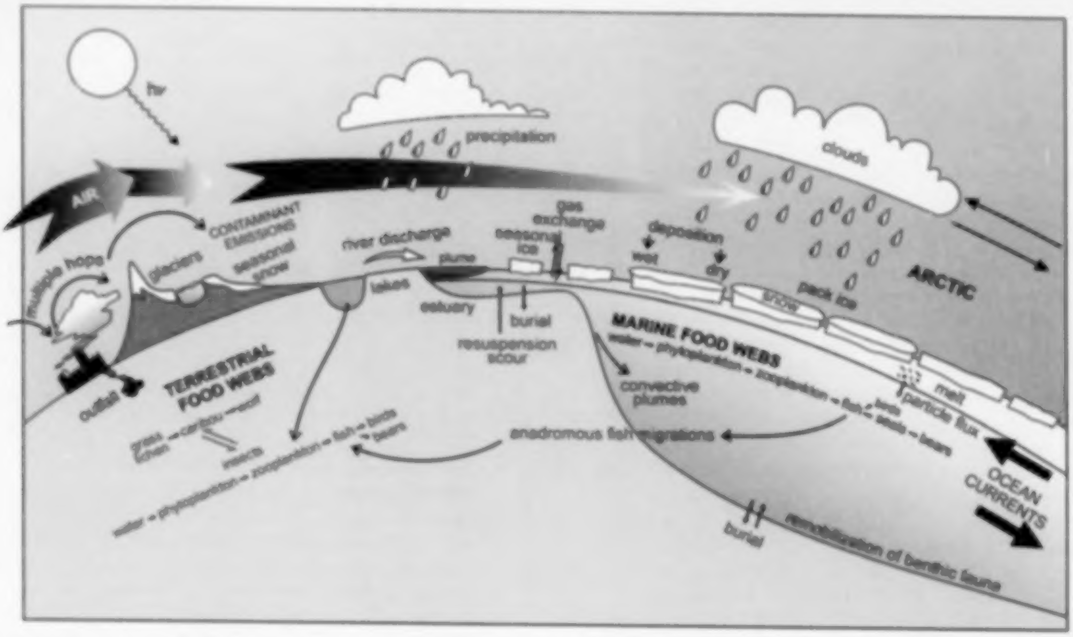
Fig. 1. Schematic drawing showing major transfers of persistent bioaccumulative toxicant (PBT) contaminants in the environment (based on Macdonald et al. 2003).


hν
AIR
precipitation
clouds
CONTAMINANT EMISSIONS
multiple hops
glaciers
seasonal snow
river discharge
plume
seasonal ice
gas exchange
deposition
wet
dry
lakes
estuary
burial
resuspension scour
outfall
TERRESTRIAL FOOD WEBS
grass lichen – caribou – wolf
insects
water – phytoplankton – zooplankton – fish – birds – bears
anadromous fish migrations
MARINE FOOD WEBS
water – phytoplankton – zooplankton – fish – birds – seals – bears
convective plumes
snow
pack ice
ARCTIC
melt
particle flux
OCEAN CURRENTS
burial
remobilization of benthic fauna

PCBs and other organochlorines in the past may continue to leach these chemicals into food webs for decades. Although new production of some of these chemicals has been banned or restricted, new releases to the environment continue. For example, active emissions of dioxins, furans, and PCBs are a concern near hazardous-waste facilities (e.g., Blais et al. 2003*a*). A recent cancer-risk assessment for dioxins, furans, PCBs, and dieldrin resulted in alerts to the public about eating farmed salmon (Hites et al. 2004).

Although the organochlorines are the most widely studied PBTs, there are many other compounds about which we know relatively little. For example, many polybrominated flame retardants, fluorinated chemicals, fragrance compounds, and current-use pesticides also have PBT potential. In a pilot screening, Environment Canada's Domestic Substance List identified 93 "new" PBT chemicals with bioconcentration factors of >5000 and half-lives in sediment of >6 months, and these were classed as inherently toxic (http://www.ec.gc.ca/substances/ese/eng/dsl/cat_index.cfm). These characteristics are comparable to those of many of the organochlorine PBTs introduced between the 1940s and 1970s.

Concentrating processes

One characteristic feature of PBTs is their ability to concentrate to high levels even in remote environments far from their origins. When a chemical is released to the environment, mixing and advection will dilute it over time and space. When chemical concentrations are higher in areas far from their original source, it implies that natural concentrating processes are active. These processes typically fall under two categories: (1) "solvent switching" and (2) "solvent reduction" (Macdonald et al. 2002). Solvent switching occurs when a chemical concentrates in one medium rather than another to move closer toward thermodynamic equilibrium. An example is when a hydrophobic chemical such as a PCB concentrates in a fish's gill because its lipid solubility greatly exceeds its aqueous solubility. Chemicals may also concentrate as a result of a solvent-depletion event, which is analogous to distillation. This category includes the enrichment of PBTs by the decomposition of organic matter, or the loss of body mass during starvation. It also includes the inefficient transfer of biomass from prey to consumer. Thus, the biomagnification of contaminants is essentially a solvent-reduction phenomenon (although bioconcentration is a solvent-switching phenomenon) (Macdonald et al. 2002). This paper explores some examples of how these chemical behaviors have profoundly affected the global distribution of PBTs.

Contamination of arctic environments

Air currents transport airborne chemicals that may be photodegraded by the sun's rays or deposited to the ground either by wet or dry deposition or by gas absorption. In addition, ocean currents move chemicals that may adsorb to particles and settle to the bottom or be incorporated into food webs (Fig. 1).

Many PBT chemicals can exhibit a remarkable capacity for long-range transport, followed by biomagnification to high concentrations in remote food chains. This was shown convincingly in the Arctic, where global transport pathways and concentrations in aquatic and marine food webs produce high exposures of predators to PBTs from their diet. In one of the earliest studies on PBTs in the north, Inuit populations from northern Quebec had higher PCB levels in maternal milk than southern populations (Dewailly et al. 1989). These results were opposite to initial expectations because Canada's remote northern environments have among the lowest population densities in the world and therefore were expected to have the lowest localized contamination. Given that PCBs and other organochlorine pesticides are only produced by human activities, it was proposed that the contamination derived primarily from long-range transport of pollutants via oceanic or atmospheric pathways. More recent analysis of organochlorine compounds in maternal milk showed that this pattern is widespread. Enrichment of pollutants in northern relative to southern populations was also recorded for dioxins, furans, DDT, dieldrin, chlordanes, and hexachlorobenzene (Gilman et al. 1997). Ingestion of these chemicals by nursing infants was shown to exceed the tolerable daily intake for adults in many cases (Gilman et al. 1997). For example, in the Yukon, the concentrations of toxaphene, a chlorinated insecticide transported through air from distant sources, were high enough in burbot (*Lota lota*) and lake trout (*Salvelinus namaycush*) that Health Canada issued a restriction of fish consumption in Lake Laberge, which had a dramatic impact on local communities (Kidd et al. 1995). These early findings prompted the instigation of the Northern Contaminants Program (http://www.ainc-inac.gc.ca/NCP/nws/nws_e.html), which is supported by Indian and Northern Affairs Canada, to study the sources, transport, fate, and effects of pollutants in northern environments. Many Canadian arctic populations, such as those on Baffin Island, show unacceptably high exposures to chemicals such as dieldrin, chlordane, toxaphene, and PCBs to this day (Indian and Northern Affairs Canada 2003). For example, between 16% and 73% of individuals in Inuit populations are presently at or above the "level of concern" for PCB exposure in places like Baffin Island, Inuvik, Kitikmeot, and Nunavut (Indian and Northern Affairs Canada 2003). Furthermore, there is evidence that polar bears suffer from deformities and reproductive impairment from PBTs (Norstrom et al. 1998).

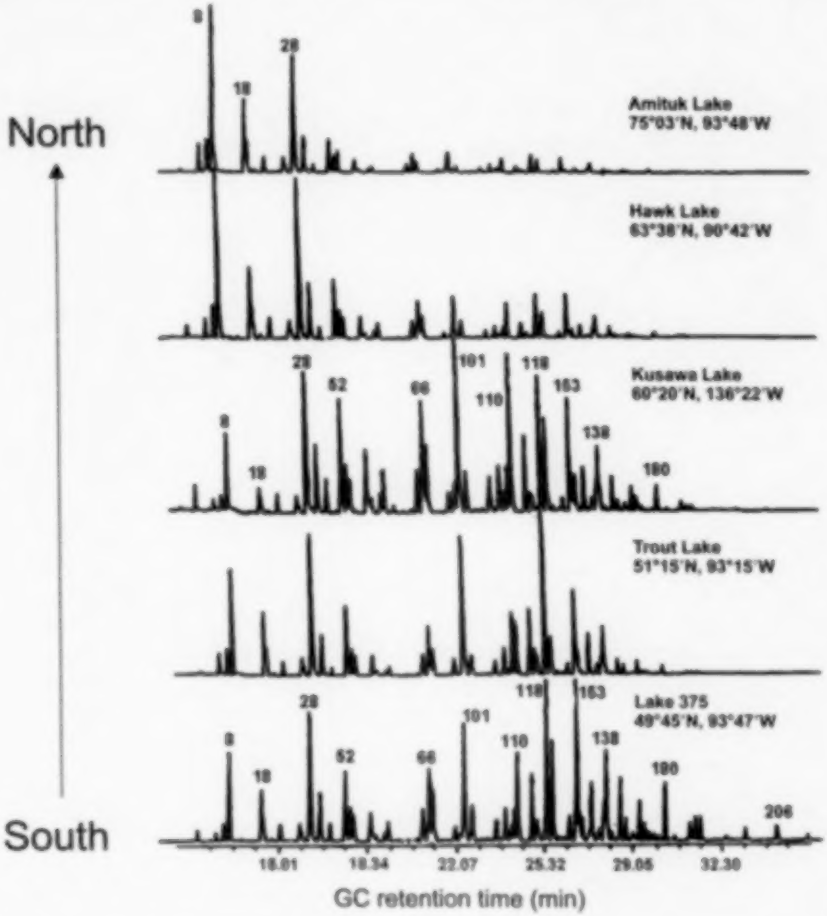
Although biomagnification of PBTs in the complex marine food web is known to be a factor in concentrating these chemicals in top predators, the presence of these chemicals at all in these distant remote sites was puzzling until the early 1990s. Wania and Mackay (1993) concluded that a global fractionation of these chemicals is taking place and predicted that it will occur such a way that more volatile compounds prevail in the more remote, colder arctic environments. In general, the global distillation model predicts that compounds with higher vapor pressures and lower condensation temperatures will tend to fractionate toward colder environments. This may happen as a result of (*i*) differential volatility at lower temperatures, causing semivolatile compounds to be retained in cold environments (solvent switching), (*ii*) funneling of semivolatile chemicals by evaporation over large tropical and temperate areas and subsequent deposition over relatively small areas in colder northern or mountain environments (solvent switching and reduction), and (*iii*) greater preservation and persistence of semivolatile chemicals in colder environments. This distillation effect has been shown to influence global distributions of many semivolatile organic contaminants. For example, Simonich and Hites (1995) showed that concentrations of the most volatile PBTs in tree bark (hexachlorocyclohexane (HCH), hexachlorobenzene, pentachloroanisole) increased significantly at higher latitudes, whereas concentrations of less volatile compounds (like DDT, endosulfan) were not correlated with latitude, which was consistent with the predictions of Wania and Mackay's (1993) global distillation model.

Further evidence in support of the global distillation model is shown by PCB-congener-specific analysis of lake sediments over a full latitude gradient across Canada (Muir et al. 1996; Fig. 2). Canadian midlatitude sediments contained a full suite of PCB congeners from the lighter, more volatile di- and tri-chlorobiphenyl congeners (e.g., nos. 8, 18, 28) to the relatively immobile nonachlorobiphenyl congeners (e.g., no. 206). A clear shift toward lower chlorinated PCBs is observed in the far north (Amituk and Hawk lakes; Fig. 2). This pattern supports the hypothesis that the heavier PCBs with higher temperatures of condensation will deposit at lower latitudes. The distribution pattern also showed a significant positive correlation between congener concentrations of PCBs 8, 18, and 28 and latitude; thus, the concentrations of these congeners are higher in the remote north than they are in the industrialized south. This outcome is consistent with predictions of the global distillation model (Wania and Mackay 1993). Studies of PCB distributions over latitudinal gradients in Canada have also identified a shift toward lower chlorinated PCB congeners in the far north from examination of burbot liver (Muir et al. 1990) and zooplankton (Koenig and Lean 1997).

When the global distillation model is allowed to run through time, Wania and Mackay (1996) suggested that there is a gradual migration of semivolatile organic compounds toward colder regions. Although chemicals like PCBs are banned in many countries and are expected to continue their decline in the industrialized parts of North America and Western Europe, their gradual migration to colder northern regions will cause them to persist well into the future. With recent declines in concentrations of persistent organochlorine pesticides in water from the Great Lakes, dates for virtual elimination, defined as a reduction to undetectable concentrations, have been estimated to range from 2010 for DDT to 2100 for hexachlorobenzene (Cortes et al. 1998). Environmental half-lives for PCBs have been estimated by Van Metre et al. (1998) at about 9 years in the southern United States with the current restrictions on PCB manufacture. However, the trend for PCBs to be deposited in lake sediments of the far north shows at least a 20-year lag (Fig. 3). These results support the predictions of the global distillation model and suggest that the problems of contamination by chlorinated xenobiotics in the far north may be prolonged. Although long-term monitoring of biota shows a slow decline in PCB and DDT concentrations in marine mammals since the 1970s (Addison and Smith 1998; Braune 1999), it is clear that the persistence of PBTs in northern biota is far greater than in biota in more southerly areas (Indian and Northern Affairs Canada 2003).

Although some PBTs like DDT and chlordane have shown declines in arctic marine animals since the 1960s, others are clearly on the rise. These PBTs include methyl mercury and brominated flame retardants called polybrominated diphenyl ethers (PBDEs; Indian and Northern Affairs Canada 2003). Levels of PBDEs are presently doubling ev-

Fig. 2. Chromatogram showing polychlorinated biphenyl (PCB) congeners in surface lake sediments over a range of latitudes. Note the enrichment of the more volatile, lower molecular weight PCBs in the north (reproduced with permission from Blais and Muir 2001). Data are from Muir et al. (1996). Numbers represent PCB nomenclature from the International Union for Pure and Applied Chemistry. GC, gas chromatograph.



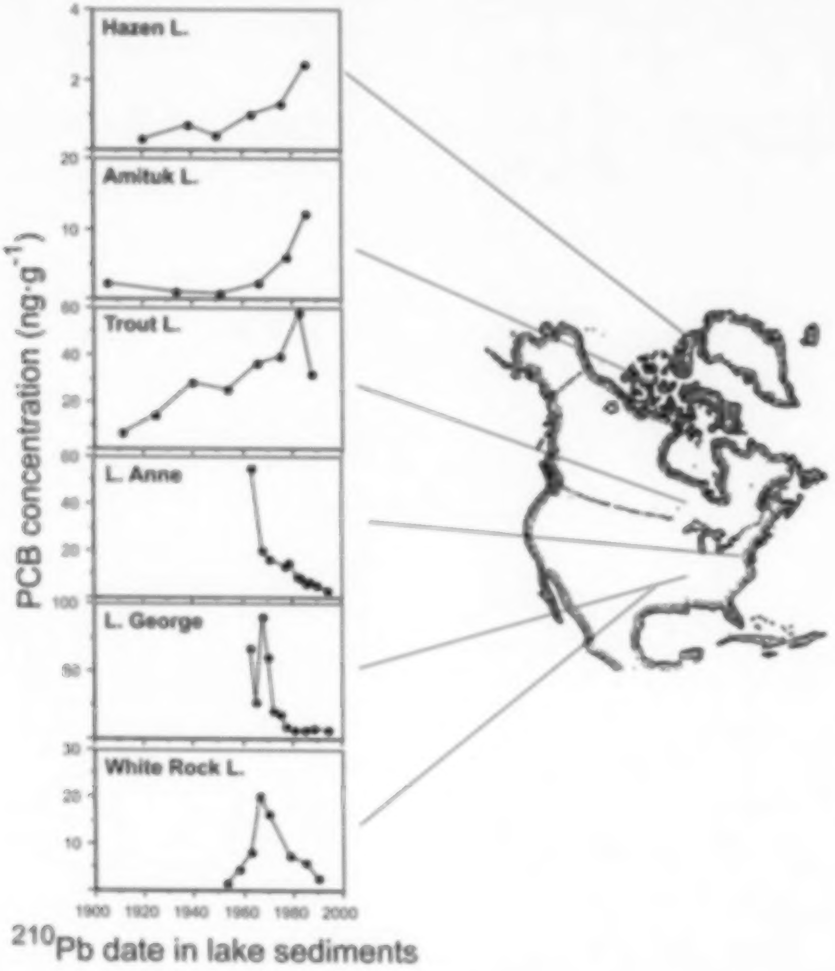
ery 4–8 years in the Canadian Arctic (Ikonomou et al. 2002), and this rise is projected to continue because of continued production and the large reservoirs of PBDEs in more southerly latitudes.

Mercury is typically included with the PBT chemicals because its organic methyl mercury form is persistent, toxic, and bioaccumulative, thereby meeting all criteria for chemicals in this class. However, distinctions must be drawn between the mercury cycle and the other organic pollutants discussed here. Elemental mercury (Hg^0) is the principal gaseous form, and its atmospheric residence time is at least 1 year (Mason and Sheu 2002), which is much longer than those of the organochlorine compounds and other persistent organic pollutants (POPs). For example, the atmospheric half-life of many organohalogen PBTs tends to be from ~30 min to 7 days (Mackay et al. 1997) because of rapid photolysis reactions in addition to oxidation by ozone, hydroxyl radicals, and nitrate radicals (Atkinson 2000). In polar regions, rapid declines in mercury concentration in the atmosphere, called mercury-depletion events, occur following polar sunrise. This photochemically induced phenomenon is thought to be the result of the oxidation of Hg^0 to Hg^{2+} by halogen radicals that are likely produced by ozone destruction following polar sunrise (Lindberg et al. 2002). These and other differences make mercury quite distinct from the other PBTs, so it should be treated as a separate entity. The interested reader should consult other more comprehensive reviews for details of the mercury cycle.

Accumulation of PBTs in alpine areas

Chemical distillation to high altitudes may be more pronounced than it is at high latitudes. For example, lower temperatures and higher precipitation, combined with close proximity to emission sources, may contribute to a greater accumulation of PBTs in alpine areas relative to lower altitudes. For example, very high rates of PBT accumulation were measured in alpine snowpacks relative to the surround-

Fig. 3. Sediment-core profiles of PCBs for a range of latitudes across North America. PCBs were first used in the 1940s and North American sales peaked in the late 1960s. Note how the northern sites show a lag in the onset and peak of PCB deposition consistent with the global distillation model (reproduced with permission from Blais and Muir 2001). Data are from Muir et al. (1996) and Van Metre et al. (1998).



ing lowlands (Blais et al. 1998), with preferential enrichment of the more volatile compounds at the highest altitudes. Greater accumulation of PBTs in soil (Ribes et al. 2002) and vegetation (Davidson et al. 2003) was also recently observed at high altitudes relative to surrounding lowlands.

Snowpack accumulation is an important contributor of PBTs to mountain lakes (Blais et al. 2001*b*), with maximum PBT loading typically occurring during the snowmelt period. Snowflakes are very effective scavengers of PBTs from the air. Once on the ground, some of these contaminants may volatilize back to the air as the snowpack matures, while those compounds with higher water solubilities (like the HCHs) tend to become dissolved in meltwater and return to the soil as the snow melts (Wania 1997). Rapid melt typically results in a pulse of contaminants to surface streams and lakes (Blais et al. 2001*a*). There is a suggestion that volatilization of some of the more volatile PBTs during snowmelt occurs as snow crystals lose their surface area during metamorphosis (solvent reduction) and may significantly increase atmospheric concentrations of PBTs during this period (Daly and Wania 2004).

In Canada, a survey of PBTs from mountain lakes showed elevated concentrations in lake trout from Bow Lake, a glacier-fed subalpine lake in Alberta near the continental divide (Donald et al. 1998). High concentrations of PBTs in lake trout from Bow Lake were not the result of pronounced biomagnification as others have shown elsewhere (Kidd et al. 1995), because these lake trout were no more than two trophic positions above grazers like gastropods (Campbell et al. 2000). A mass flux analysis of PBTs in Bow Lake revealed that a large majority of contaminants entering this lake are from glaciated tributaries that receive high snowfall every year (Blais et al. 2001*b*). Another important source was contaminants in the air, where deposition and retention by lakes is favored at low temperatures and high precipitation. Glaciers appear to act as effective funnels for POPs, likely because glacial runoff in summer becomes channelized on the glacier surface and is routed rapidly to surface waters. Therefore, cold glacial meltwater has limited contact with soils and sediments, leaving little opportunity for losses of PBTs by evaporation and binding to organic-poor glacial sediments (Blais et al. 2001*a*, 2001*b*). A pulse in radioactive hydrogen (tritium) in glacial melt in late summer also provided evidence that these meltwaters contain precipitation from decades of higher atmospheric bomb testing (1960s and 1970s), when many PBTs such as HCHs and PCBs were also higher in precipitation (Blais et al. 2001*a*).

Alpine and subalpine drainage basins have limited plant biomass, particularly above the treeline, and shallower soil development than lower altitudes. Most PBTs bind strongly to organic matter, thus thin soils and sparse vegetation might favor the transfer of contaminants from alpine catchments to streams and lakes. In high-altitude lakes, low primary productivity and low dissolved organic carbon (DOC) are often observed (Donald et al. 1998), which could make PBTs more bioavailable to flora and fauna.

Meteorological factors further affect contaminant distributions in mountain lakes. Years of high snowfall coincide with years of high concentrations of pesticides in lakes (Wilkinson et al. 2005). Calculations of air–water gas exchange in lakes spanning a 1200-m range of elevations showed that the magnitude and direction of air–water gas exchange were not significantly correlated with site elevation or temperature (Wilkinson et al. 2005). These results showed that air–water gas exchange in mountain lakes is a very dynamic process and highly sensitive to seasonal and interannual fluctuations in temperature and in PBT concentrations in water and air. Wilkinson et al. (2005) also showed that air concentrations of HCHs are declining and causing a reversal in the direction of gas exchange from net deposition to volatilization in these mountain lakes, similar to what has occurred in the Bering and Chukchi seas (Jantunen and Bidleman 1995).

Enrichment of contaminants in food webs of alpine lakes relative to lakes at lower altitudes has been observed, but the causes are not entirely clear. Toxaphene concentrations in fish correlated with lake elevation (Donald et al. 1998), suggesting that either physical or biological factors were causing concentration of PBTs at high altitudes. Grimalt et al. (2001) showed that PCBs and other organochlorine-pesticide concentrations in fish correlated strongly with elevation in lakes across Europe, and Vives et al. (2004) extended this analysis to include the effects of age and condition factor. Feeding behavior further impacts accumulation of PBTs in mountain lakes. In Bow Lake, pelagic-feeding lake trout accumulated more PBTs than littoral feeders (Campbell et al. 2000). The likely contributor of contamination to the pelagic food web in this lake was the lipid-rich copepods (*Hesperodiaptomus arcticus*), which accumulated PBTs to high concentrations.

Several other biological factors might favor the accumulation of PBTs in alpine food webs. Alpine fauna have shown slower growth rates and sometimes store more lipids than those at lower altitudes, both factors favoring higher concentrations of PBTs (Thomann 1989). Amphipods (*Gammarus lacustris*) in alpine lakes had lower growth rates than amphipod populations at lower altitudes, which contributed to higher concentrations of PBTs in these animals (Blais et al. 2003*b*).

Biotransport of contaminants by migratory animals

Another interesting feature of PBTs is their capacity to be transported in significant quantities by migratory animals. Chemicals with a high capacity for biomagnification concentrate in animal flesh, which in some cases can become an important vector for transporting contaminants along migratory pathways. Several examples of this phenomenon exist.

PBTs in migratory salmon

Most Pacific salmon species are anadromous. The life cycle of Pacific salmon (*Oncorhynchus* spp.) begins in fresh waters, where they will spend at least one winter. As small juvenile fish, they migrate to salt water and acquire 99% of their adult biomass. Upon maturity, salmon return to their natal stream or lake to spawn and then die. Nutrients and contaminants accumulated during their marine existence are transported back to their freshwater nursery lakes and streams, where they are released. In some lakes, salmon-derived nutrients can make up to 60% of the annual total phosphorus nutrient load (Koenings and Burkett 1987). Preliminary studies have shown that anadromous fish may be important vectors for POPs (e.g., PCBs and organochlorine pesticides, methyl mercury) to freshwater systems (Ewald et al. 1998). In one study, it was shown that Arctic grayling (*Thymallus arcticus*, resident fish) from a lake that supported sockeye salmon (*Oncorhynchus nerka*) were significantly more contaminated with PCBs than grayling from a lake that did not support sockeye salmon (Ewald et al. 1998). They also observed that the contaminants in grayling from the two lakes were very different in composition. The grayling from the lake that received anadromous salmon were enriched in many of the more bioaccumulative POPs like the heavier PCBs and DDT, which were shown to be concentrated in the returning salmon (Ewald et al. 1998), implying that the salmon were the source of contaminants. This pathway was confirmed by Krümmel et al. (2003), who found that PCB delivery to lake sediments was closely correlated with salmon spawner density in eight Alaskan lakes and that high salmon returns boosted the rate of PCB delivery to lakes by 7 times the amount in lakes that do not receive salmon spawners.

Migrating salmon are also important vectors for methyl mercury. Zhang et al. (2001) estimated that sockeye salmon have transported over 20 kg of methyl mercury to Bristol Bay (Alaska) rivers from the ocean over the past 20 years. Recently, Sarica et al. (2004) showed that freshwater chinook salmon (*Oncorhynchus tshawytscha*) likewise deliver significant quantities of total mercury, methyl mercury, and nutrients to a Lake Ontario spawning stream following their spawning, death, and decomposition. Aquatic and terrestrial invertebrates feeding on salmon had 25× more mercury than those that were not exposed to salmon carcasses. There were clear correlations between salmon densities and water concentrations of mercury, DOC, and NH_4^+, indicating that the salmon spawning cycle dramatically affects the delivery of contaminants and nutrients to spawning areas in Lake Ontario tributaries.

Biotransport of PBTs by seabirds

Seabirds may also be an important vector for PBTs to freshwater lakes. Evenset et al. (2004) compared two lakes on Bear Island (Norway). Lake Ellasjøen is a coastal lake with a major seabird population and Lake Øyangen is an inland lake with less precipitation and no seabirds. Very high concentrations of PCBs and other organochlorine compounds were observed in the fish and plankton of Ellasjøen relative to Øyangen, which suggested that seabirds may be transporting contaminants to lakes via their guano. The PCB concentrations measured in fish from Ellasjøen were 10–40 times higher than those from Øyangen (Evenset et al. 2004), and significant CYP450 induction was measured in fish from Ellasjøen, showing that PBT contaminants are inducing a toxic response in fish from Ellasjøen (Matt Vijayan, Department of Biology, University of Waterloo, Waterloo, ON N2L 3G1, personal communication). These results suggest that seabirds deliver contaminants to the lakes where they nest, and this contamination has an impact on aquatic food webs.

In summary, PBTs are among the most pervasive substances that humans have ever created. There is virtually no place on Earth that is untouched by these chemicals. As a society we have created contaminated areas in places far from where the chemicals were ever used. We must appreciate that there are natural processes concentrating these chemicals to levels that can cause physiological harm. For example, chemicals may be distilled to cold environments and reach levels in arctic and high-altitude areas that are high enough to trigger consumption advisories. Chemicals may also be funneled and translocated by migratory species. The organochlorines have been studied most extensively, but there are many other PBTs about whose distribution, fate, and effects in the environment we have no information.

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Other information regarding submission

Cover letter

The corresponding author should send a cover letter with the submission, signed by all authors, that

(*i*) states the main points and significance of the work;

(*ii*) avows that all coauthors fully participated in and accept responsibility for the work;
(*iii*) avows that the manuscript is not being considered for publication elsewhere;
(*iv*) suggests potential referees;
(*v*) identifies other manuscripts, including "extended abstracts", containing the same, similar, or related information, which other manuscripts may be generated;
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Each manuscript is normally submitted to two referees for appraisal. However, the Senior Editor will return unreviewed those manuscripts that do not fall within the Journal's scope or character, and those that exceed the Journal's guidelines for prior publication as "extended abstracts" (guidelines available from the Editorial Office's Web site at http://www.uoguelph.ca/~cjfas/). Papers submitted for inclusion in supplements are treated with the same rigor of review as articles in regular issues.

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Duplicate and prior publication

It has long been a policy of the *Canadian Journal of Fisheries and Aquatic Sciences* to not publish manuscripts that have been published elsewhere. The Senior Editor considers a paper not eligible for publication if most of the content of the paper (*i*) is under consideration for publication or is published in a journal, or book chapter; (*ii*) is under consideration for publication or is published in a conference proceedings or a government publication, with a substantial circulation (distributed to 100 or more individuals over a wide area). Authors may place a draft of a submitted article on their Web site or their organization's server, provided that the draft is not amended once accepted for publication. We encourage authors to insert hyperlinks from preprints to the final published version on the NRC Research Press Web site (http://pubs.nrc-cnrc.gc.ca). Abstracts or extended abstracts related to conferences do not constitute prior publication. Extended abstracts are usually under 2000 words and do not include presentation of detailed tables and graphics of the results of the study. Further guidelines on extended abstracts are available from the Editorial Office Web site (http://www.uoguelph.ca/~cjfas/).

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In the cover letter, the corresponding author must affirm that all of the authors have contributed substantially to the manuscript and approved the final submission.

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Before writing any of the manuscript, list tentative headings in as few ranks as possible. Rework them until they appear to allow logical development for the reader; usually, chronological order is not effective. The findings will be more readily appreciated if methods, findings, and discussion are given in separate sections.

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Begin sections and paragraphs with topic sentences containing generalizations that lead readily to the particulars. Giving a conclusion first and then supporting it not only improves readability but also facilitates assessment by other scientists. Failure to give the most newsworthy generalizations first is one of the most prominent shortcomings in presentation of manuscripts.

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An abstract is required for every contribution and should contain accurate descriptive words that will draw the reader to the content. This is particularly important because contemporary alerting services and search engines will search this text. It should not be more than 175 words and should appear on a separate page. The concise abstract should present the paper content accurately and should supplement, not duplicate, the title in this respect. Authors able to submit abstracts in both fluent English and French are encouraged to do so. Abstracts submitted in one language will be translated into the other official language by the journal translator. References should not be cited in the abstract unless they are absolutely essential, in which case full bibliographic information must be provided.

Like the title, the abstract enables readers to determine the paper's content and decide whether they need to read the entire paper. Begin the abstract with the main conclusion from the study, and support it with the relevant findings. Limit details of methods to those needed in understanding what was done and work them into statements of findings. Avoid using phrases such as "…is discussed" or "…was found"; be specific. As the abstract is often divorced from the main body of the paper by abstracting and indexing services and is the only part of a paper some readers ever see, it is important that it accurately reflect the paper's contents and be completely self-contained (i.e., any *essential* references) in a retrievable form (e.g., R.B. Deriso. 1980. Can. J. Fish. Aquat. Sci. **37**: 268–282).

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Limit the introduction largely to the scope, purpose, and rationale of the study. Restrict the literature review and other background information to that needed in defining the problem or setting the work in perspective. Try beginning with the purpose or scope of the work, defining the problem next, and adding guideposts to orient the reader. An introduction generally need not exceed 375–500 words.

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Materials and methods provides the framework for getting answers to the questions posed in the purpose of the work.

Limit the information on materials and methods to what is needed in judging whether the findings are valid. To facilitate assessment, give all the information in one section when possible. Refer to the literature concerning descriptions of equipment or techniques already published, detailing only adaptations. Often, it helps to begin statements on procedures with a phrase indicating the purpose, such as "To determine…we…". If the section is long, consider using subheadings corresponding to headings for the findings.

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Limit the Discussion to giving the main contributions of the study and interpreting particular findings, comparing them with those of other workers. Emphasis should be maintained on synthesis and interpretation and exposition of broadly applicable generalizations and principles. If these are exceptions or unsettled points, note them and show how the findings agree or contrast with previously published work. Limit speculation to what can be supported with reasonable evidence. End the Discussion with a short summary of the significance of the work and conclusions drawn. If the Discussion is brief and straightforward, it can be combined with the Results section.

Acknowledgements

Acknowledgements should be written in the third person. We strongly urge authors to limit acknowledgments to those who contributed substantially to scientific and technical aspects of the paper, gave financial support, or improved the quality of the presentation. Avoid acknowledging those whose contribution was clerical only.

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Footnotes to material in the text should not be used unless they are unavoidable, but their use is encouraged in tables. Where used in the text, footnotes should be cited in the manuscript by superscript Arabic numbers (except in the tables, see below) and should be numbered serially beginning with any that appear on the title page. Each footnote should be typed on the manuscript page upon which the reference is made; **footnotes should not be included in the list of references**.

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References

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The author is responsible for verifying each reference against the original article. Each reference must be cited in the text using the surnames of the authors and the year, for example, (Walpole 1985) or Green and Brown (1990). Depending on the sentence construction, the names may or may not be in parentheses, but the year always is. If there are three or more authors, the citation should give the name of the first author followed by et al. (e.g., Green et al. 1991). If references occur that are not uniquely identified by the authors' names and year, use *a*, *b*, *c*, etc., after the year, for example, Green 1983*a*, 1983*b*; Green and Brown 1988*a*, 1988*b*, for the text citation and in the reference list.

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References to unpublished data, manuscripts in preparation or submitted to other journals, progress reports, and unpublished papers given at annual meetings are not cited in the reference list but may be included as footnotes or in parentheses in the text, giving all authors' names and initials. For a private communication, year of communication should also be given (e.g., J.S. Jones (personal communication, 1999)). If consultants' reports or other documents of limited circulation must be cited, they should carry with them an availability statement explaining where the document can be obtained. If an unpublished book or article has been **accepted for publication**, include it in the reference list followed by the notation "In press". Do not include volume and page number in an in-press reference, as these are subject to change before publication.

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References should be selected judiciously and be largely restricted to significant, published literature. References should follow the form used in current issues of the Journal. The names of serials are abbreviated in the form given in *Chemical Abstracts Service Source Index* (*CASSI*) (Chemical Abstracts Service, 2540 Olentangy River Road, P.O. Box 3012, Columbus, OH 43210-0012, USA) or in *BIOSIS Serial Sources* (BIOSIS, 2100 Arch Street, Philadelphia, PA 19103-1399, USA). In doubtful cases, authors should write the name of the serial in full. The Journal encourages the inclusion of issue numbers which should be placed in parentheses after the volume number. The following bibliographic citations illustrate the punctuation, style, and abbreviations for references.

Examples of reference types, including electronic references

Journal article:

Campana, S.E., and Thorrold, S.R. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations. Can. J. Fish. Aquat. Sci. **58**(1): 30–38.

Entire issue of journal:

Gordon, D.C., Jr., and Hourston, A.S. (*Editors*). 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. **40**(Suppl. 1).

Report:

Sanders, W.W., Jr., and Elleby, H.A. 1970. Distribution of wheel loads in highway bridges. National Cooperative Highway Research Program Report 83, Transportation Research Board, National Research Council, Washington, D.C.

Book:

Williams, R.A. 1987. Communication systems analysis and design. Prentice-Hall, Inc., Englewood Cliffs, N.J.

Book in a series:

Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. No. 184.

Part of book:

Healey, M.C. 1980. The ecology of juvenile salmon in Georgia Strait, Britsh Columbia. *In* Salmonid ecosystems of the North Pacific. *Edited by* W.J. McNeil and D.C. Himsworth. Oregon State University Press, Corvallis, Oreg. pp. 203–229.

Paper in conference proceedings:

Whittaker, A.A., Uang, C.-M., and Bertero, V.F. 1990. Experimental seismic response of steel dual systems. Proceedings of the 4th U.S. National Conference on Earthquake Engineering, Palm Springs, Calif., Vol. 2, pp. 655–664.

Institutional publications and pamphlets:

Dzikowski, P.A., Kirby, G., Read, G., and Richards, W.G. 1984. The climate for agriculture in Atlantic Canada. Available from the Atlantic Advisory Committee on Agrometeorology, Halifax, N.S. Publ. ACA 84-2-500. Agdex No. 070.

Corporate author:

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1975. Standard methods for the examination of water and wastewater. 14th ed. American Public Health Association, American Water Works Association, and Water Pollution Control Federation, Washington, D.C.

Thesis:

Keller, C.P. 1987. The role of polysaccharidases in acid wall loosening of epidermal tissue from young *Phaseolus vulgaris* L. hypocotyls. M.Sc. thesis, Department of Botany, The University of British Columbia, Vancouver, B.C.

Electronic citation:

Quinion, M.B. 1998. Citing online sources: advice on online citation formats [online]. Available from http://clever.net/quinion/words/citation.html [cited 20 October 1998].

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Newbury, M.G., and Ashworth, A.C. 2004. A fossil record of colonization and response of lacustrine fish populations to climate change. Can. J. Fish. Aquat. Sci. **61**(10): 1807–1876. doi: 10.1139/F04-113

Translation:

Koike, A., and Ogura, B. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. **64**: 1–11. [Translated from Japanese by Can. Transl. Fish. Aquat. Sci. 4950, 1983.]

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General

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Bitmap (raster) files are image files produced using a grid format in which each square (or pixel) is set to one level of black, colour, or grey. A bitmap (rasterized) file is broken down into the number of pixels or picture elements per inch (ppi). Pixels per inch is sometimes referred to as dots per inch (dpi). The higher the resolution of an image, the larger the number of pixels contained within the rectangular grid. The proper resolution should be used when submitting bitmap artwork. The minimum requirements for resolution are 600 dpi for line art, 1200 dpi for finelines (line art with fine lines or shading), 300 dpi for halftones and colour, and 600 dpi for combinations (halftones with lettering outside the photo area).

Colour

All colour files submitted must be as CMYK (cyan, magenta, yellow, and black). These colours are used in full-colour commercial printing. RGB graphics (red, green, and blue; colours specifically used to produce an image on a monitor) will not print correctly.

Vector

Vector files are image files produced using elements such as lines and shapes. Typically these files are used for line drawings.

Bitmap in vector

Bitmaps can be imported into vector/draw applications only for the purpose of adding and overlaying information, lines, text, etc. Bitmaps should not be resized, cropped, rotated, or otherwise manipulated after importing.

Manuscript guidelines

Style guides

As a general guide for biological terms, *The CBE Manual for Authors, Editors, and Publishers: Scientific Style and Format* (6th ed., 1994) published by the Council of Biology Editors, Inc., Chicago, IL 60603, USA, is recommended.

Spelling

Spelling should follow *Webster's Third New International Dictionary* or the *Oxford English Dictionary*. Authors are responsible for consistency in spelling.

Nomenclature, abbreviations, and acronyms

Nomenclature and abbreviations should follow the rules recommended by the International Union of Biochemistry (IUB)

Committee of Editors of Biochemical Journals with support of IUPAC. As a general guide for biological terms, *The CBE Manual for Authors, Editors, and Publishers: Scientific Style Format* (6th ed., 1994), published by the Council of Biology Editors, Inc., Chicago, IL 60603, USA, is recommended. For enzyme nomenclature, *Enzyme Nomenclature (1992): Recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology* (Academic Press, San Diego, Calif.) should be followed.

Abbreviations and acronyms that are standard in the discipline need not be defined. All others must be defined when they are first mentioned in the text and those with more than one meaning should be avoided.

Units of measurement

SI units (Système international d'unités) should be used or SI equivalents should be given. Avoid ambiguous forms such as g $C/m^2/day$; use g $C{\cdot}m^{-2}{\cdot}day^{-1}$. This system is explained and other useful information is given in the *Metric Practice Guide* (2000) published by CSA International (178 Rexdale Blvd., Toronto, ON M9W 1R3, Canada). For practical reasons, some exceptions to SI units are allowed. Units such as kilocalorie, reciprocal centimetre (wave number), and atmosphere may be used for the foreseeable future.

Statistical analyses

The assumptions and (or) the model underlying any statistical analysis should be clearly stated. Symbols such as * and **, denoting levels of significance, should not be used except in conjunction with the actual values of the associated test statistic; actual *p* values are preferred.

Fish

The Journal follows the names and spelling for fishes recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (5th ed., Spec. Publ. No. 20, American Fisheries Society, 1991) and the gene nomenclature for protein-coding loci outlined in Shaklee et al. (Trans. Am. Fish. Soc. **119**: 2–15, 1990).

Writing numbers

In writing long numbers the digits should be separated into groups of three, counted from the decimal marker to the left and right. The separator should be a space and not a comma, period, or any other mark, for example, 25 562 987 and not 25,562,987. In English text, the decimal marker should be a point, for example, 0.1 mL and not 0,1 mL. The decimal point in all numbers between 1 and −1, except 0, must be preceded by a 0. The sign × should be used to indicate multiplication, e.g., 3×10^6 and not $3{\cdot}10^6$.

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Recommandations aux auteurs

Journal canadien des sciences halieutiques et aquatiques

Orientation du Journal et directives de soumission de manuscrits

La Rédaction encourage la soumission d'articles contribuant à l'enrichissement des connaissances des sciences halieutiques et aquatiques. Les articles peuvent porter sur les cellules, les organismes, les populations, les communautés, les écosystèmes ou les procédés qui affectent les systèmes aquatiques. Ils peuvent traiter de disciplines variées y compris la biologie et l'écologie des organismes d'eau douce et d'eau salée, la limnologie, l'océanographie, la physiologie, la toxicologie, la génétique, l'économie, les maladies et la gestion. Les articles sont choisis selon la portée et l'importance des nouvelles connaissances ou idées qu'ils contiennent. On accorde la préséance aux articles qui contribuent à la compréhension de phénomènes observés et à l'interprétation de résultats expérimentaux.

Nous invitons les auteurs à soumettre des articles ayant des objectifs précis ou relevant des hypothèses, concepts ou questions vérifiables et se terminant par de solides conclusions ou d'utiles synthèses. Ces articles enrichissent, changent, mettent en question ou réorientent les connaissances acquises sur divers aspects des sciences aquatiques. **La raison d'être de l'analyse et l'interprétation des résultats doivent être situées dans un vaste contexte disciplinaire ou interdisciplinaire.** Les articles de méthodologie et de modélisation doivent inclure des applications et prouver la supériorité de la méthodologie ou du modèle explicité.

La Rédaction décourage la soumission d'articles simplement descriptifs (à moins qu'il ne s'agisse de nouvelles disciplines), d'application très limitée (applicable à une seule année, une seule région ou un seul taxon, portant sur un unique composé chimique, etc.); d'articles qui ne font que reprendre les principes énoncés dans des articles précédents; ou d'articles rendant compte de l'application de normes sans gain de méthodologie. Les études préliminaires ou fragmentaires, ou dont la pertinence à grande échelle n'est pas démontrée, et les interprétations purement spéculatives sont carrément rejetées.

Les articles doivent être aussi complets que possible. S'il est impossible d'inclure toute les données pertinentes dans un seul article, l'auteur devra inclure des renvois à des articles connexes et soumettre ceux-ci avec son propre article.

Types de manuscrits

Le Journal publie le genre de textes suivants :

Articles — Études de vaste portée qui constituent de nouvelles contributions à la science.

Perspectives — Synthèses, synthèses critiques ou réévaluations de concepts ou de paradigmes couramment admis.

Discussions — Commentaire ou réplique sur des sujets récemment abordés dans le Journal.

Communications rapides — Présentations de nouveaux concepts, descriptions de nouvelles méthodologies ou courts textes sur des sujets d'actualité ou controversés.

On peut obtenir les caractéristiques précises de chacun de ces genres sur le site Web du Bureau de la rédaction (http://www.uoguelph.ca/~cjfas/).

Langue

Les manuscrits doivent être rédigés de manière claire et concise, **en français ou en anglais correct**. Les auteurs pour qui le français ou l'anglais est une langue seconde devraient faire réviser leur manuscrit par une personne dont la langue maternelle est celle du texte avant de soumettre leur manuscrit. Un style de qualité accélère et facilite l'examen et la publication des manuscrits.

Un résumé rédigé dans la langue du manuscrit doit accompagner chaque soumission. Les résumés sont traduits en anglais ou en français, selon le cas, par le traducteur du Journal. En revanche, les auteurs qui peuvent soumettre leur résumé en français et en anglais sont invités à le faire.

Frais d'impression par page

Il n'y a pas de frais d'impression par page dans le *Journal canadien des sciences halieutiques et aquatiques*.

Objectif des recommandations

Pour **faciliter la publication**, les auteurs doivent vérifier l'exactitude, l'uniformité et la lisibilité des symboles, des abréviations et des termes techniques. Les Presses scientifiques du CNRC se réservent le droit d'assurer la qualité technique du Journal. Les auteurs voudront consulter un récent numéro du Journal pour s'enquérir des exigences en matière de présentation, en particulier à l'égard des tableaux et de la bibliographie. **La non-conformité des manuscrits et des illustrations aux exigences décrites ci-dessous risque de retarder la publication.**

Soumission

Soumission électronique

La Rédaction **encourage les auteurs à soumettre leurs manuscrits par courriel** à cjfas@uoguelph.ca en format Word, WordPerfect ou PDF. Pour obtenir de plus amples renseignements sur la soumission électronique, y compris les formats de fichier acceptés, communiquez directement avec le Bureau de la rédaction.

Soumission par courrier

Comme alternative, les auteurs devraient soumettre par

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courrier **leurs manuscrits (y compris les tableaux et les illustrations) en triple** à la directrice scientifique principale, *Journal canadien des sciences halieutiques et aquatiques*, Université de Guelph, Département de biologie, écologie et évolution des organismes, édifice 006, bureau 107, Guelph, ON N1G 2W1, Canada. Veuillez inclure le texte, les tableaux et les figures du manuscrit sur disquette, en précisant le(s) progiciel(s) et la (les) version(s) utilisés; le logiciel de traitement de textes préférés est Microsoft Word pour **l'examen par les réviseurs**.

Renseignements complémentaires sur la soumission

Lettre de présentation

L'auteur-correspondant doit accompagner son manuscrit d'une lettre de présentation, signée par tous les auteurs, qui fait état :

(*i*) des principaux points et de l'importance du travail;
(*ii*) de la participation de tous les auteurs au manuscrit et de leur approbation de ce dernier;
(*iii*) de l'assurance que le manuscrit en question n'est pas en cours d'examen aux fins de publication dans une autre revue;
(*iv*) de suggestions quant aux arbitres éventuels;
(*v*) d'autres manuscrits, y compris de résumés détaillés, contenant une information identique ou similaire à celle contenue dans le manuscrit soumis;
(*vi*) des numéros de téléphone et de télécopieur ainsi que du courriel et de l'adresse de chaque auteur;
(*vii*) de tout conflit d'intérêts, réel ou perçu (cf. « Conflit d'intérêts et divulgation » sous la rubrique « Éthique »).

Formulaires de cession des droits d'auteur

Le manuscrit doit être accompagné de formulaires de cession des droits d'auteur dûment signés par tous les auteurs (cf. « Cession des droits d'auteur » sous la rubrique « Processus de publication »).

Emballage

L'emballage des manuscrits et des illustrations devrait être assez solide pour prévenir les dommages dus au transport.

Processus de révision

Réception des manuscrits

Un accusé de réception sera envoyé à l'auteur-correspondant suivant l'arrivée du manuscrit. La conformité du manuscrit aux *Recommandations aux auteurs* sera vérifiée par un lecteur technique. Les manuscrits non conformes pourront être renvoyés à l'auteur-correspondant pour que la présentation soit corrigée avant que le contenu n'en soit examiné.

Examen par les pairs/évaluation

Chaque article est habituellement soumis à une évaluation par deux réviseurs. Toutefois, la directrice scientifique principale renverra sans les évaluer tout article qui ne reflète pas la portée ou le caractère du Journal et les articles qui débordent des limites imposées pour la publication sous forme de « résumés approfondis ». On obtiendra les directives à cet égard sur le site Web du Bureau de la rédaction à http://www.uoguelph.ca/~cjfas/. Les articles soumis aux fins de publication dans un supplément sont soumis au même rigoureux exercice d'examen que les articles des numéros réguliers.

On demande habituellement aux auteurs d'examiner les commentaires des réviseurs et de modifier leur texte en conséquence en 90 jours, tout au plus. Faute de respecter ce délai, l'article renvoyé par l'auteur sera traité comme une nouvelle soumission à moins que l'auteur n'ait au préalable dûment communiqué avec le Bureau de la rédaction.

Manuscrits acceptés

Les auteurs sont priés de soumettre **une copie imprimée ainsi qu'une copie électronique (sur disquette ou par courriel) seulement de la version définitive du manuscrit accepté**. Si la copie électronique est présentée sur disquette, les fichiers de texte et les fichiers d'illustration doivent être soumis sur des disquettes distinctes. Le nom des auteurs, le nom et la version du logiciel utilisé de même que la plate-forme (IBM ou Macintosh) doivent être clairement indiqués sur chaque disquette. Le **texte** (y compris les tableaux) doit être soumis en format de traitement de texte (de préférence WordPerfect, Microsoft Word ou TeX, compatible IBM ou Macintosh). Des macros TeX pour la préparation des manuscrits sont disponibles en direct à ftp://ftp.tex.ac.uk/tex-archive/macros/latex/contrib/nrc/, ftp://ftp.dante.de/tex-archive/macros/latex/contrib/nrc/ et ftp://ctan.tug.org/tex-archive/macros/latex/contrib/nrc/. Quant aux **illustrations**, il convient de se reporter à la section « Préparation des fichiers graphiques électroniques ». Tout manuscrit devra être accompagné d'une lettre faisant foi du fait que la version du texte sur disquette reflète exactement la version imprimée définitive.

Manuscrits resoumis

Les auteurs qui soumettent un manuscrit précédemment refusé ou retiré doivent donner le numéro de manuscrit de leur première soumission. Les manuscrits resoumis sont traités comme de nouveaux manuscrits. Les auteurs peuvent soumettre le nouveau manuscrit par courriel en format Word, WordPerfect ou PDF, accompagné d'une réponse détaillée et systématique à toutes les questions soulevées au cours de l'évaluation précédente. Comme alternative, les auteurs peuvent soumettre l'original et deux copies du nouveau manuscrit par courrier, accompagnés d'une lettre de réponse détaillée.

Processus de publication

Généralités

La Rédaction vérifie tous les articles soumis afin de s'assurer qu'ils respectent les *Recommandations aux auteurs* et qu'ils sont accompagnés de toute la documentation d'appui nécessaire. Tout élément posant problème sera examiné par la Rédaction en consultation avec l'auteur-correspondant. Une fois que tous les problèmes sont résolus par la Rédaction et que tous les formulaires de cession des droits d'auteur dûment signés par les auteurs sont reçus, le manuscrit est acheminé aux Presses scientifiques du CNRC, à Ottawa, en vue de sa publication. La préparation finale des manuscrits est assurée par un lecteur-rédacteur responsable de l'uniformité de la présentation et du style.

Correspondance avec les Presses scientifiques du CNRC

Après qu'un article a été accepté, toute correspondance doit être adressée aux Presses scientifiques du CNRC, Conseil national de recherches du Canada, Ottawa, ON K1A 0R6, Canada (télécopieur : 613-952-7656; courriel : pubs@nrc-cnrc.gc.ca; URL : http://pubs.nrc-cnrc.gc.ca). Les Presses scientifiques du CNRC se réservent le droit d'apporter des modifications rédactionnelles mais ne modifieront pas la substance d'un article sans préalablement consulter l'auteur et la directrice scientifique principale.

Épreuves en placard

Une épreuve en placard, les épreuves d'illustration, une copie de l'article annoté et un bon de commande de tirés à part seront envoyés à l'auteur-correspondant. **Les épreuves en placard doivent être relues attentivement car elles ne seront pas revues par les Presses scientifiques du CNRC.** Elles doivent en outre être renvoyées dans les 48 heures suivant leur réception. Au stade des épreuves, il n'est plus temps d'apporter des modifications importantes ni de faire des ajouts ou des suppressions. Les frais occasionnés par des modifications excessives devront être assumés par les auteurs.

Tirés à part

Pour obtenir des tirés à part, il convient de remplir le bon de commande à cet effet, d'y annexer le paiement pertinent (chèque, numéro de carte de crédit, numéro de bon d'achat ou pièce de journal interne) et de renvoyer le tout en même temps que les épreuves et l'article corrigés. Le tarif des tirés à part commandés après la publication est fortement majoré. **Le Journal n'offre pas de tirés à part gratuits. Les tirés à part commandés ne sont postés qu'après réception d'un numéro du bon d'achat ou du paiement pertinent.**

Permission de reproduction de documents protégés par droits d'auteur

Les auteurs d'un manuscrit qui contient de la documentation déjà publiée (tableaux, illustrations, schémas, etc.), et donc assujettie aux droits d'auteur, doivent obtenir **une permission écrite, de la part du titulaire de ces droits d'auteur**, de reproduire l'information en question **en format imprimé et électronique**. Les permissions écrites doivent accompagner le manuscrit soumis, sans quoi le processus de publication pourra être retardé. Cette lettre sera exigée avec toute mention du type « Tirée de… ». Si une illustration est modifiée, de sorte qu'une telle autorisation n'est pas requise, la formulation doit être explicit : « Modifiée d'après… ». Si l'illustration est reconstituée à partir d'informations tirées d'autres auteurs la mention doit être du même type que pour une référence.

Cession des droits d'auteur

Tous les auteurs doivent remplir un formulaire de cession des droits d'auteur au CNRC. Les auteurs peuvent obtenir ces formulaires auprès de la directrice scientifique principale, dans le premier numéro d'un volume ou sur le site Web du Journal (http://pubs.nrc-cnrc.gc.ca/cgi-bin/ps/rp2_auth_f?cjfas).

Permission de republication de documentation publiée dans les revues des Presses scientifiques du CNRC

Toute demande de permission de reproduction ou de republication partielle ou intégrale d'un manuscrit doit être adressée à Roxanne Landriault, des Presses scientifiques du CNRC (613-990-2254; courriel : roxanne.landriaultn@nrc-cnrc.gc.ca).

Éthique

Généralités

Les normes d'éthique auxquelles doivent se conformer les auteurs, les examinateurs et les directeurs scientifiques sont décrites dans la Politique éditoriale des Presses scientifiques du CNRC (publiée dans le numéro de janvier 1996 du *Journal canadien des sciences halieutiques et aquatiques*, diffusée sur le site Web du Journal à http://pubs.nrc-cnrc.gc.ca/cgi-bin/ps/rp2_cust_f?pubpolicy ou disponible auprès des Presses scientifiques du CNRC).

Reproduction et rediffusion

En vertu d'une politique de longue date, le *Journal canadien des sciences halieutiques et aquatiques* refuse de publier des articles qui ont été publiés ailleurs. La directrice scientifique principale juge un manuscrit inadmissible si le manuscrit en question (*i*) est en cours d'examen en vue d'être publié ou a déjà été publié dans un périodique ou dans un livre, (*ii*) est en cours d'examen en vue d'être publié ou a déjà été publié dans un compte rendu de conférence ou dans une publication officielle ouvertement distribuée à plus de 100 exemplaires. Les auteurs peuvent proposer, sur leur site Web ou sur le serveur de leur institution, une ébauche d'un article soumis, à condition que l'ébauche ne soit aucunement modifiée après l'acceptation du manuscrit. Les auteurs sont invités à inclure, sur la version préimprimée de leur manuscrit, un lien hypertexte vers sa version définitive sur le site Web des Presses scientifiques du CNRC (http://pubs.nrc-cnrc.gc.ca). Les résumés concis ou détaillés inclus dans les comptes rendus de conférences ne constituent pas une « publication antérieure ». Un « résumé détaillé » est un document de 2000 mots ou moins qui ne comprend ni tableaux détaillés ni illustrations des résultats de l'étude. Des renseignements supplémentaires sur les résumés détaillés sont disponibles sur le site Web du Bureau de la rédaction (http://www.uoguelph.ca/~cjfas/).

Déclaration de la paternité du manuscrit

Dans sa lettre de présentation, l'auteur-correspondant doit garantir que les auteurs ont tous contribué de près au manuscrit et ont approuvé la version soumise.

Suggestion d'examinateurs

Les auteurs peuvent suggérer des examinateurs. Ils doivent fournir l'adresse complète, les numéros de téléphone et de télécopieur, ainsi que l'adresse de courriel (dans la mesure du possible) de ces personnes. La directrice scientifique principale n'est aucunement tenus de se limiter à ces suggestions.

Conflit d'intérêts et divulgation

La directrice scientifique principale reconnaît la possibilité que des conflits d'intérêts réels ou perçus surviennent à la suite de circonstances intellectuelles, personnelles ou financières liées aux travaux de recherche. Les manuscrits doivent inclure une divulgation de toute source de financement de la recherche. De plus, la lettre de présentation doit expliquer tout conflit d'intérêts, réel ou perçu, qui pourrait survenir lors du processus d'examen par les pairs. Si de tels conflits ne sont pas divulgués, le manuscrit pourrait être refusé.

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Retouche photographique

Le Journal estime que les photographies numériques font partie des données. Aussi est-il impératif que les images numériques soumises reproduisent fidèlement l'image d'origine. Toute retouche à l'aide d'un logiciel graphique doit être explicitée dans les méthodes, notamment quant au logiciel utilisé et aux techniques appliquées pour améliorer ou modifier l'illustration. Une telle explication assure la constance des méthodes et l'intégrité scientifique des travaux. La suppression des artéfacts ou de toute donnée non essentielle contenue dans l'image est découragée.

Sections du manuscrit

Disposition et organisation

Le manuscrit doit être composé à **double interligne**, d'un seul côté de feuilles de 21,5 sur 28 cm (8,5 po sur 11 po ou ISO A4). Chaque page doit être numérotée, en commençant par la page de titre. Le texte destiné à être publié en italique doit être présenté en italique, non pas souligné. Les auteurs utiliseront des caractères en majuscule seulement lorsque cela s'avèrera pertinent.

Les auteurs devraient structurer le manuscrit en fonction de l'énoncé des objectifs ou de la portée de l'étude présentée dans l'introduction. Il est particulièrement important que le titre et les en-têtes reflètent l'énoncé des objectifs.

Avant de commencer la rédaction du manuscrit, les auteurs devraient dresser une liste provisoire des en-têtes, avec le moins de subdivisions possible, et remanier cette liste jusqu'à ce qu'elle reflète une démarche logique, intelligible pour le lecteur. Habituellement, l'ordre chronologique ne sera pas satisfaisant. En effet, il sera plus facile de suivre le texte si les méthodes, les résultats et la discussion font partie de sections distinctes.

Les auteurs devraient organiser les tableaux et les illustrations de manière à faciliter les comparaisons et regrouper les données apparentées de façon à réduire au minimum le nombre de tableaux et d'illustrations. Dans la mesure du possible, les tableaux et les figures devraient être suffisamment explicites pour que le lecteur n'ait pas à se reporter au texte.

Les auteurs devraient commencer les sections et les paragraphes par des énoncés généraux qui aboutissent naturellement à des énoncés pointus. La présentation de la conclusion dans un premier temps et de sa justification dans un deuxième facilite considérablement la lecture et l'évaluation par les scientifiques. Lors de la rédaction des manuscrits, le défaut le plus courant consiste à omettre de présenter dès le début du texte les généralités les plus dignes de mention.

Les auteurs doivent veiller à ce que le contenu de chaque section soit pertinent à l'en-tête et que le contenu de chaque paragraphe se rapporte à la première phrase du paragraphe (la « phrase d'ouverture »).

Avant de rédiger les paragraphes, les auteurs devraient composer la phrase d'ouverture de chaque paragraphe et disposer chacune de celles-ci dans l'ordre approprié.

Titre

Les auteurs doivent limiter le titre à ce que démontre le manuscrit. Le titre est un élément fondamental de l'article et doit refléter clairement et brièvement le contenu du manuscrit. Le titre a deux fonctions : (*i*) il permet au lecteur de juger si, oui ou non, l'article est susceptible de l'intéresser et (*ii*) il devrait fournir suffisamment d'information pour permettre au lecteur de juger de la portée et de l'importance potentielle du contenu. Les mots du titre devraient fournir un maximum d'information et identifier la nature de la recherche, de l'organisme utilisé et, le cas échéant, la démarche technique (p. ex. radiographie, chromatographie, analyse mathématique). Les titres ne devraient pas commencer par un chiffre ou par des prépositions d'introduction telles que « Sur » ou « Vers », ou des expressions telles que « Contribution à… » ou « Recherche sur… ». Des titres judicieux facilitent considérablement le classement des publications et leur utilisation par les scientifiques et les bibliothécaires et de même que leur dépouillement par les indexateurs. Les titres en série devraient être évités.

Page de titre

La page de titre doit comprendre les suivants. (*i*) Le titre intégral du manuscrit. (*ii*) Le nom des auteurs, dans l'ordre dans lequel ces noms doivent apparaître dans l'intitulé de l'article imprimé. (*iii*) L'affiliation et l'adresse (y compris l'adresse de courriel) de chaque auteur pendant les recherches. Les changements d'affiliations et d'adresses (y compris d'adresse de courriel) devront être indiqués en note infrapaginale. (*iv*) Le nom, l'adresse, les numéros de téléphone et de télécopieur ainsi que l'adresse de courriel de l'auteur-correspondant.

Nom des auteurs

La directrice scientifique principale encourage tous les auteurs à fournir leur prénom au complet plutôt qu'une simple initiale.

Résumé

Chaque contribution doit être accompagnée d'un résumé qui utilisera un vocabulaire évocateur, susceptible de capter l'intérêt des lecteurs visés. Ceci est particulièrement important en vue du dépouillement automatisé que font les services de repérage de l'information de même que les robots des moteurs de recherche. Un résumé comptera au plus 175 mots et sera présenté sur une page distincte. Le résumé concis doit refléter précisément le contenu du manuscrit et doit compléter le titre plutôt que simplement le reprendre. Les résumés sont traduits en anglais ou en français, selon le cas, par les traducteurs du Journal. En revanche, les auteurs qui peuvent soumettre leur résumé en français et en anglais sont invités à le faire. Le résumé ne devrait pas contenir de références à moins que cela ne soit essentiel. Dans ce dernier cas, il faudra fournir l'information bibliographique intégrale.

À l'instar du titre, le résumé permet au lecteur de prendre connaissance du contenu du manuscrit et de décider si, oui ou non, il lui faut le lire en entier. Les auteurs devraient présenter, au début du résumé, la principale conclusion de l'étude et l'appuyer par les résultats pertinents. Ils ne doivent donner que les détails des méthodes nécessaires à la compréhension de la démarche et les intégrer à l'énoncé des résultats. Il faut éviter l'emploi de phrases telles que « … on y analyse » ou « … a été trouvé » et être plus spécifique. Comme le résumé est souvent séparé du corps principal de l'article par les services d'analyse et les indexateurs et qu'il s'agit parfois de la seule partie de l'article que certains lecteurs auront l'occasion de voir, il importe qu'il reflète précisément le contenu du manuscrit et qu'il soit complet en lui-même, c.-à-d. qu'il contienne seulement des références absolument essentielles et facilement retraçables (p. ex. R.B. Deriso. 1980. Can. J. Fish. Aquat. Sci. **37** : 268–282).

Introduction

Les auteurs doivent limiter l'introduction à la portée, au but et à la raison de l'étude. Ils ne mentionneront que les travaux publiés et autres documents nécessaires à la définition du problème et du contexte. Les auteurs devraient tout d'abord décrire le but ou la portée de l'étude, définir ensuite le problème puis présenter des repères qui orienteront le lecteur. En général, une introduction comprendra de 375 à 500 mots.

Matériel et méthodes

La section « Matériel et méthodes » jette les fondations de la résolution du problème posé dans l'énoncé du travail.

Les auteurs limiteront les renseignements sur le matériel et les méthodes à ce qui est nécessaire pour juger de la validité des résultats. Cette évaluation sera facilitée si toute l'information connexe est présentée, autant que possible, dans une même section. S'il s'agit d'outillage ou de techniques connus, les auteurs incluront des références aux publications et ne décriront en détail que les adaptations. Il y a souvent avantage à commencer un énoncé des méthodes par une phrase qui en indique le but, comme : « Afin de déterminer... nous... ». Si la section est longue, les auteurs devraient la diviser à l'aide d'en-têtes correspondant aux en-têtes de la section des résultats.

Résultats

Les auteurs doivent limiter les résultats aux réponses apportées aux questions en rapport avec l'énoncé de travail et faire en sorte que ces réponses soient aussi complètes que possible tout en étant concises. Les résultats doivent être associés aussi étroitement que possible aux observations ou aux mesures, ceci afin que le lecteur ne confonde pas constations et spéculations. Les auteurs devraient dégager les principales constations mises en évidence par les tableaux et les illustrations et éviter de répéter dans le texte ce que les légendes expriment clairement. La documentation complémentaire au texte peut faire partie de la documentation du rapport intégral ou être versée dans un dépôt de données reconnu. Les auteurs peuvent y faire référence dans le texte (cf. section « Données supplémentaires »).

Discussion

Les auteurs devraient limiter la discussion à la présentation des principales contributions de l'étude et à l'interprétation de constatations particulières, en les comparant avec celles d'autres auteurs. Ils mettront l'accent sur la synthèse, l'interprétation et la présentation de généralisations et de principes largement applicables. S'il existe des exceptions ou des aspects douteux, les auteurs le souligneront et feront ressortir la manière dont les résultats corroborent ou contredisent les travaux déjà publiés. La spéculation doit se limiter à ce qui peut être appuyé sur des preuves raisonnables. Les auteurs devraient terminer cette section par un sommaire de l'importance du travail et par les conclusions qu'il a invitées. Si la discussion est brève et précise, elle peut être combinée avec les résultats en une seule section.

Remerciements

Les remerciements sont rédigés à la troisième personne. Les auteurs sont priés de limiter les remerciements aux personnes qui ont étroitement contribué aux aspects scientifiques et techniques de l'article, qui ont fourni une aide financière ou qui ont sensiblement amélioré la qualité de la présentation. Les remerciements ne devraient pas viser les fournisseurs de service de soutien.

Notes infrapaginales

Les notes infrapaginales ne devraient figurer dans le corps du texte que lorsqu'elles sont indispensables; toutefois, leur utilisation dans les tableaux est encouragée. Lorsque des notes infrapaginales figurent dans le texte, les appels devraient être signalés à l'aide d'un chiffre arabe en exposant (sauf dans le cas des tableaux, cf. ci-dessous) et numérotés de façon consécutive à partir de la première note infrapaginale, y compris toute note apparaissant en page titre. Chaque note infrapaginale doit figurer au bas de la page où elle est signalée, **pas dans la bibliographie**.

Équations et liste de symboles

Les **équations** doivent être clairement dactylographiées et, si elles incluent des exposants ou des indices, être dactylographiées à triple interligne. Les exposants et les indices doivent être lisibles et correctement placés. Il convient de rendre évidente la distinction entre la lettre *l* et le chiffre *un*, et entre la lettre majuscule *O* et le chiffre *zéro*. Une lettre ou un symbole ne devrait être associé qu'à une seule réalité et devrait être utilisé de façon uniforme dans l'article. Chaque variable doit être définie dans le texte ou dans une **liste des symboles** qui succédera à la bibliographie. Les variables représentant des vecteurs, des matrices, des matrices vectorielles et des tenseurs doivent être clairement définies. Les équations doivent être identifiées à l'aide de chiffres arabes placés entre parenthèses, alignés avec la **marge de gauche**. Aucune distinction n'est faite entre la numérotation des formules mathématiques et celle des formules chimiques.

Références

Présentation générale

Il appartient aux auteurs d'assurer l'adéquation entre le contenu des références bibliographiques et l'information bibliographique des publications citées. Dans le texte, les références doivent être signalées à l'aide du nom des auteurs et de l'année de publication, p. ex. (Martial 1970) ou Dupont et Tremblay (1990), selon la structure de la phrase. Lorsqu'il y a trois auteurs ou plus, le nom du premier auteur est suivi de la mention et al. (p. ex. Dupont et al. 1991). Lorsque le nom et l'année ne suffisent pas à distinguer deux ouvrages, les lettres *a*, *b*, *c*, etc. sont ajoutées après l'année (à la fois dans le texte et dans la bibliographie), p. ex. Dupont 1983*a*, 1983*b*; Dupont et al. 1980*a*, 1980*b*).Les adresses Web (adresses URL) ou les identificateurs d'objets numériques (système DOI) permettent de consulter les références qui sont accessibles sur le Web. Les auteurs sont invités à les utiliser et à les inclure avec les références dans la bibliographie (voir exemple ci-dessous).

Rapports non publiés, communications personnelles et documents sous presse

Les références bibliographiques à des rapports non publiés, à des communications personnelles ou à des articles en cours d'examen ne paraîtront pas dans la bibliographie. Elles seront plutôt signalées sous forme de notes infrapaginales ou entre parenthèses dans le texte. Elles devront contenir le nom des auteurs et leur initiale. Dans le cas des communications personnelles, il convient de préciser la nature de la source et l'année (p. ex. J.S. Ernst (communication personnelle, 1999)). Si l'on doit citer des rapports d'experts-conseils ou d'autres

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documents dont la distribution est limitée, on devra préciser l'endroit où l'on peut se les procurer. Si un livre ou un article non publié avéré est **en attente de publication**, sa référence doit apparaître dans la bibliographie et porter la mention « sous presse ». Dans ce cas, il ne faut pas préciser le volume et le numéro de la page, puisque cette information risque de changer lors du processus de publication.

Présentation de la bibliographie

Dans la **bibliographie**, qui apparaît à la suite du texte, les références sont composées à double interligne et classées par ordre alphabétique du nom du premier auteur, sans numérotation. Si la bibliographie comprend plusieurs publications d'un même premier auteur, les règles suivantes s'appliquent : (*i*) les articles avec **un seul auteur** sont signalés en premier, par ordre chronologique, en commençant par le plus ancien; (*ii*) viennent ensuite les articles avec **deux auteurs**, en ordre alphabétique du nom de famille du second auteur; (*iii*) puis viennent les articles de **trois auteurs et plus**, en ordre chronologique.

Directives générales quant aux références

On choisira les références avec soin, les limitant aux publications importantes. Les références doivent refléter le style des numéros récents du Journal. Le titre d'une revue doit être abrégé conformément au *Chemical Abstracts Service Source Index* (*CASSI*) (Chemical Abstracts Service, 2540 Olentangy River Road, P.O. Box 3012, Columbus, OH 43210-0012, É.-U.) ou à *BIOSIS Serial Sources* (BIOSIS, 2100 Arch Street, Philadelphia, PA 19103-1399, É.-U.). En cas de doute, les auteurs doivent écrire le nom de la revue au complet. Nous recommandons aux auteurs de préciser le numéro des revues citées et de l'indiquer entre parenthèses après le numéro du volume. La liste de références qui suit reflète les normes de ponctuation, de présentation et d'abréviation préconisées.

Exemples de divers types de références, y compris les références à des documents électroniques

Article de revue :

Archambault, D., et Bourget, E. 1983. Importance du régime de dénudation sur la structure et la succession des communautés intertidales de substrat rocheux en milieux subarctiques. Can. J. Fish. Aquat. Sci. **40**(8) : 1278–1292.

Numéro intégral d'une revue :

Gordon, D.C., Jr., et Hourston, A.S. (*directeurs de la rédaction*). 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. **40** (Suppl. 1).

Rapport :

Pigeon, M., et Langlois, M. 1987. Étude de la durabilité au gel d'un ciment avec fumée de silice. Ciments Saint-Laurent, Beauport (Québec). Rapp. GCT-87-09.

Livre :

Lacroix, P., et Lalonde, J. (*directeurs de la rédaction*).1992. Les grands barrages. Éditions Poly-Sciences, Montréal (Québec).

Livre faisant partie d'une collection :

Scott, W.B., et Crossman, E.J. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. n° 184.

Section d'un livre :

Healey, M.C. 1980. The ecology of juvenile salmon in Georgia Strait, British Columbia. *Dans* Salmonid ecosystems of the North Pacific. *Sous la direction de* W.J. McNeil et D.C. Himsworth. Oregon State University Press, Corvallis (Oregon). p. 203–229.

Article d'un compte rendu de conférence :

Milot, D. 1973. Le barrage de Montreuil. *Dans* Les grands barrages. *Sous la direction de* F. Wang et C. Dulude. 2^e Symposium international sur les barrages, Chicoutimi (Québec), 8 au 10 mai 1972. Éditions P. Lacoste, Montréal (Québec). p. 102–150.

Publication ou brochure d'institution :

Dzikowski, P.A., Kirby, G., Read, G., et Richards, W.G. 1984. The climate for agriculture in Atlantic Canada. Disponible auprès du Atlantic Advisory Committee on Agrometeorology, Halifax (Nouvelle-Écosse). Publ. ACA 84-2-500. N° Agdex 070.

Auteur collectif :

American Public Health Association, American Water Works Association et Water Pollution Control Federation. 1975. Standard methods for the examination of water and wastewater. 14^e éd. American Public Health Association, American Water Works Association et Water Pollution Control Federation, Washington (District de Columbia).

Mémoire :

Keller, C.P. 1987. The role of polysaccharidases in acid wall loosening of epidermal tissue from young *Phaseolus vulgaris* L. hypocotyls. Mémoire de maîtrise ès sciences, Département de botanique, The University of British Columbia, Vancouver (Colombie-Britannique).

Document électronique :

Quinion, M.B. 1998. Citing online sources: advice on online citation formats [en ligne]. Disponible à http://clever.net/quinion/words/citation.html [cité le 20 octobre 1998].

Référence comprenant une adresse Web ou un système DOI :

Newbury, M.G., et Ashworth, A.C. 2004. A fossil record of colonization and response of lacustrine fish populations to climate change. Can. J. Fish. Aquat. Sci. **61**(10) : 1807–1876. doi: 10.1139/F04-113

Traduction :

Koike, A., et Ogura, B. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. **64**: 1–11. [Traduit du japonais par Can. Transl. Fish. Aquat. Sci. 4950, 1983.]

Tableaux

Les tableaux, qui occuperont chacun une page distincte, doivent suivre la bibliographie, être numérotés de façon continue en chiffres arabes dans l'ordre de mention dans le manuscrit et comporter un bref titre descriptif, d'au plus une phrase, intelligible sans qu'il ne soit nécessaire de se reporter au texte. Les titres de colonne doivent être brefs, mais peuvent être étoffés à l'aide de notes infratabulaires. Les tableaux ne doivent pas contenir de traits verticaux. Les auteurs voudront consulter un exemplaire du Journal pour s'enquérir de la présentation souhaitable des tableaux. Les notes infratabulai-

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res doivent être signalées par des symboles (selon l'ordre suivant : *, †, ‡, §, ‖, ¶, #) ou des lettres minuscules en italique placées en exposant. Une note descriptive qui n'est pas introduite par un appel de note peut être inscrite au bas du tableau à titre de **Nota**. Les auteurs éviteront d'insérer de nombreux petits tableaux et garderont le nombre de tableaux au minimum.

Légendes des illustrations

Les légendes des illustrations doivent constituer une liste **sur une page distincte**, qui suivra les tableaux. Les légendes devraient être descriptives de sorte qu'il ne soit pas nécessaire de se reporter au texte. Elles ne devraient pas inclure d'information expérimentale : celle-ci aura plutôt été présentée comme il se doit dans la section « Méthodes ». Dans les graphiques, les légendes ne doivent pas reprendre le label des axes mais plutôt décrire ce que révèlent les données. Dans le cas de montages d'illustrations, les auteurs rédigeront une légende globale contenant suffisamment d'information pour décrire l'ensemble du montage. Si chaque élément mérite sa propre légende, le montage sera fractionné et les illustrations seront plutôt présentées de façon individuelle.

Appendices

Un appendice est un document distinct et complet. Les illustrations et les tableaux présentés dans les appendices doivent être numérotés de façon consécutive, distinctement des illustrations et tableaux du corps de l'article, p. ex. figure A1, tableau A1, etc. Si les appendices contiennent des références bibliographiques, ces dernières doivent apparaître dans une bibliographie distincte de la biographie du corps de l'article. S'il y a plus qu'un appendice, veuillez les identifier de la façon suivante : Appendice A, Appendice B, etc.

Données supplémentaires

Le Conseil national de recherches du Canada administre un dépôt des données non publiées dans lequel les données supplémentaires peuvent être versées, à la demande des auteurs ou de la directrice scientifique principale. De plus, il est possible d'accéder à ces données dans leur format d'origine à partir du site Web du Journal grâce aux hyperliens à cet effet apparaissant sur la version Web de l'article. Les données supplémentaires peuvent comprendre de grands tableaux, des calculs complexes et des cartes détaillées qui ne sont pas essentiels à la compréhension ou à l'évaluation du document. Les auteurs doivent clairement indiquer les parties dont ils demandent le dépôt dès la soumission de leur manuscrit. Les tableaux et les illustrations doivent être numérotés de façon consécutive, distinctement de ceux destinés à la publication (p. ex. : figure S1, tableau S1). Chaque ensemble de données supplémentaires doit faire l'objet d'une note infrapaginale. Des copies des données déposées peuvent être obtenues contre paiement auprès du Dépôt des données non publiées, ICIST, Conseil national de recherches du Canada, Ottawa, ON K1A 0R6, Canada.

Programmes informatiques

La Rédaction ne publie habituellement pas les imprimés détaillés produits à l'aide de programmes informatiques. Si les données contenues dans un tel imprimé contribuent sensiblement à la valeur de l'article, l'auteur doit mettre deux copies du programme en dépôt (cf. section « Données supplémentaires »).

Illustrations

Généralités

Les auteurs doivent soumettre les illustrations originales et deux copies des illustrations au Bureau de la rédaction, même si seul le fichier électronique est destiné à servir à la publication.

Chaque illustration ou groupe d'illustrations doit être préparé de manière à occuper, après réduction, l'espace d'une ou deux colonnes du texte. Les dimensions définitives maximales d'une illustration sur une colonne sont de 8,6 sur 23,7 cm (3,4 sur 9,3 po) et celles d'une illustration sur deux colonnes de 18,2 sur 23,7 cm (7,2 sur 9,3 po). Les illustrations (y compris les similis) sont numérotées en chiffres arabes de façon consécutive. Elles doivent toutes être signalées dans l'article et être intelligibles sans qu'il ne soit nécessaire de se reporter au texte. Tous les termes, les abréviations et les symboles qu'elles contiennent doivent être identiques à ceux qui apparaissent dans le texte. Seuls les détails essentiels devraient être identifiés; l'information détaillée devrait figurer dans la légende. Chaque **illustration soumise sur papier** doit être identifiée, au bas, à gauche ou encore au verso de l'illustration, par le numéro de l'illustration et le nom des auteurs, mentions qui seront suffisamment éloignées de l'espace réservé à l'illustration.

Dessins au trait

L'épaisseur des traits doit être suffisante (au moins 0,5 point) pour que la reproduction soit nette; tous les symboles, les exposants, les indices et les signes décimaux doivent être proportionnés au reste du dessin et d'une dimension suffisante pour permettre la réduction nécessaire sans perte de détail. Il convient de ne pas utiliser de petits symboles ouverts, qui ont tendance à se refermer lorsqu'ils sont reproduits. **Les caractères d'imprimante à points, de machine à écrire ou écrits à la main ne sont pas acceptables.** Les illustrations de dimensions semblables doivent afficher le même style de police et la même dimension de caractère. Les imprimés originaux des **spectromètres RMN, IR, RPE,** etc. ne sont pas acceptables. Ils doivent être retracés au propre. Les **dessins au trait originaux soumis sur papier** doivent être faits à l'encre noire ou produits par ordinateur à l'encre noire sur du papier blanc de première qualité ou sur un support comparable. Quant aux dessins produits par ordinateur, il convient de fournir un imprimé au laser de la meilleure résolution disponible.

Cartes

Les cartes doivent avoir des **motifs clairs et gras**, et comporter les longitudes et latitudes (ou les coordonnées de Mercator transverses universelles) de même qu'une échelle graphique, ceci afin de pouvoir repérer avec exactitude l'emplacement du travail. Sur **les cartes du Québec**, le nom officiel des municipalités doit être utilisé (p. ex. Québec, Montréal, Clarke City). De plus, le nom des éléments topographiques doit systématiquement être en français (p. ex. Lac Bienville), à l'exception des noms d'intérêt pancanadien. En effet, ces noms ont une version officielle française et anglaise (p. ex. « Océan Atlantique » et « Atlantic Ocean ») et doivent donc être présentés dans la langue de l'article. La province de Québec, dans les cartes en anglais, doit être désignée « Quebec ». Les auteurs trouveront une liste complète des noms des régions d'intérêt pancanadien dans *Le guide du rédacteur*

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(p. 236–237, 2e édition, 1996), publié par Travaux publics et Services gouvernementaux Canada, Ottawa, ON K1A 0S5.

Photographies

Les photographies doivent être en ton continu, de première qualité, avec contrastes bien marqués, rognées de façon à ne laisser paraître que les éléments essentiels. Une photographie ou un groupe de photographies doit être préparé de manière à occuper, **sans réduction supplémentaire**, une ou deux colonnes du texte. Les micrographies électroniques et les photomicrographies doivent comporter une échelle graphique à même le cliché. Pour assurer un bon résultat de la reproduction, les auteurs uniformiseront le contraste et la densité de toutes les photographies montées sur une même planche. Les **photographies soumises sur papier** doivent être imprimées sur papier glacé. Elles doivent être rognées et montées sur carton bristol blanc **mince**. Les photographies en montage doivent être juxtaposées sans espace.

Illustrations en couleur

Les illustrations en couleur sont reproduites aux frais de l'auteur. Les auteurs qui désirent en savoir davantage doivent communiquer avec la rédactrice administrative du Journal, Cecily Pearson (613-993-9099; télécopieur : 613-952-7656; courriel : cecily.pearson@nrc-cnrc.gc.ca).

Préparation des fichiers graphiques électroniques

Généralités

Les Presses scientifiques du CNRC préfèrent recevoir les fichiers électroniques des graphiques des manuscrits acceptés et utiliseront ces fichiers dans la mesure du possible.

Si les fichiers électroniques ne sont pas disponibles ou ne se prêtent pas à la reproduction, les Presses feront le balayage optique des graphiques imprimés de première qualité soumis par les auteurs ou sollicités auprès de ceux-ci. Il importe de mentionner que le balayage optique fait ressortir les défauts (p. ex. liquide correcteur, macules). Les auteurs sont priés de ne pas soumettre de photographies en ton non continu (tramées) ni d'illustrations balayées puis imprimées au laser. En effet, la reproduction de ces documents cause souvent des moirures, c.-à-d. un effet visuel indésirable résultant du rebalayage ou du retramage d'une illustration formée de points.

Si les auteurs soumettent des originaux imprimés, les fichiers électroniques doivent y correspondre sur le plan de la numérotation et du contenu. Les auteurs qui soumettent une disquette doivent indiquer sur son étiquette (*i*) l'application et la version du logiciel et (*ii*) le nom, la dimension et le suffixe des fichiers. Si les fichiers ont été comprimés, les auteurs doivent préciser le format de compression. Les versions PC ou Macintosh des polices True Type ou Type 1 doivent être utilisées. **Les auteurs ne doivent pas se servir des polices de caractères en mode point ou non standard.** Les graphiques électroniques peuvent être soumis sur disquette de 3 po ½, sur cartouche Zip de 100 Mo ou sur cédérom.

Le logiciel graphique que préconisent les Presses scientifiques du CNRC est CorelDraw! Pour connaître les applications compatibles, les auteurs peuvent se reporter à la liste des programmes graphiques électroniques à http:// pubs.nrc-cnrc.gc.ca/cgi-bin/ps/rp2_prog_f?jees_graphics_f.html.

Toutes les illustrations doivent être soumises dans leur dimension de tirage final. Quant aux illustrations comportant plusieurs parties (p. ex., *a*, *b*, *c*, *d*, etc.) crées sur le même logiciel, elles doivent être regroupées dans un seul fichier plutôt que transmises individuellement.

Il convient de garder les illustrations le plus simple possible afin d'éviter les complications. Les auteurs voudront éviter les textures et les fonds grisés compliqués, particulièrement dans les programmes d'illustration vectorielle, puisque ceci risque d'altérer la qualité des illustrations au moment du tirage.

Fichiers en mode point

Les fichiers d'images en mode point sont produits selon une grille dans laquelle chaque carré (ou pixel) correspond à une nuance de noir, de couleur ou de gris. Un fichier en mode point (ou de données tramées) est défini par le nombre de pixels ou d'éléments d'image par pouce. Comme le pixel est défini comme un point, on parle aussi de « points par pouce » ou « ppp ». Plus la résolution de l'image est élevée, plus le nombre de points sur une surface donnée est élevé. Les illustrations soumises en mode point doivent être de résolution suffisante. Les exigences minimales de résolution sont les suivantes : 600 ppp pour les dessins au trait, 1200 ppp pour les traits fins (les dessins au trait comportant des traits fins ou un fond grisé), 300 ppp pour les similis et les couleurs et 600 ppp pour les combinaisons (similis avec caractères à l'extérieur de l'espace réservé à la photo).

Fichiers en couleur

Tous les fichiers en couleur soumis doivent être en mode couleur CMJN (cyan, magenta, jaune et noir). Ces couleurs sont utilisées pour l'impression commerciale en polychromie. Les graphiques RVB (rouge, vert et bleu), soit le mode couleur pour la production d'images à l'écran, ne s'impriment pas correctement.

Fichiers vectoriels

Les fichiers vectoriels sont des fichiers d'images composées d'éléments tels que des traits et des formes. Ce type de fichier est habituellement utilisé pour les dessins au trait.

Mode point dans les fichiers vectoriels

Les éléments en mode point peuvent être importés dans un logiciel vectoriel ou de dessin pour ajouter ou superposer de l'information, des traits, du texte, etc. Les éléments d'image en mode point ne doivent pas être redimensionnés, recadrés, tournés ou manipulés de quelque façon après avoir été importés.

Directives pour les manuscrits

Guides de style

Il est recommandé d'utiliser, comme guide général dans le domaine de la biologie, *The CBE Manual for Authors, Editors, and Publishers : Scientific Style and Format* (6e édition, 1994), publié par le Council of Biology Editors, Inc. (Chicago, IL 60603, É.-U.).

Orthographe

L'orthographe doit respecter celle du *Petit Robert*. Les auteurs sont responsables de l'uniformité de l'orthographe dans l'ensemble du texte. Les accents doivent être insérés sur les majuscules.

Nomenclature, abréviations et acronymes

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